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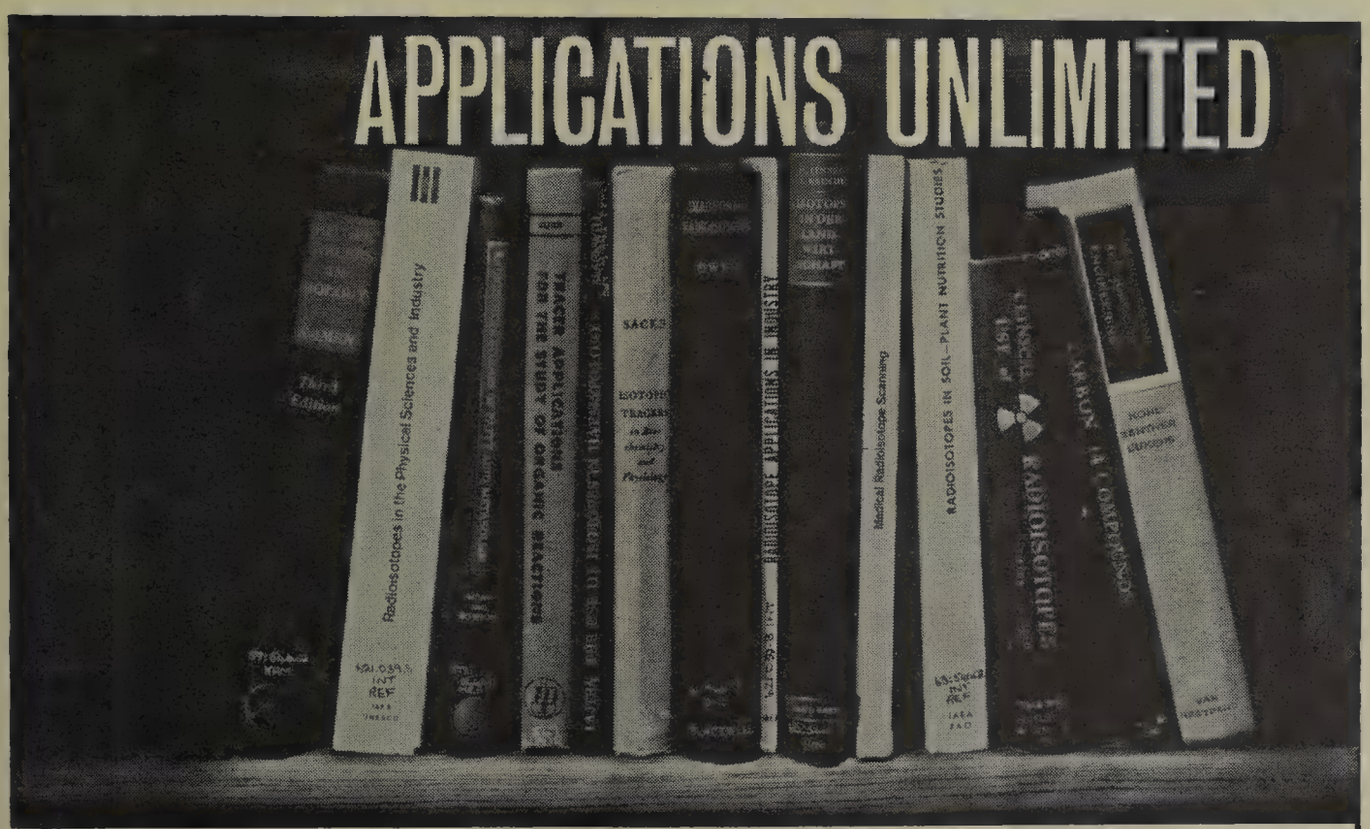
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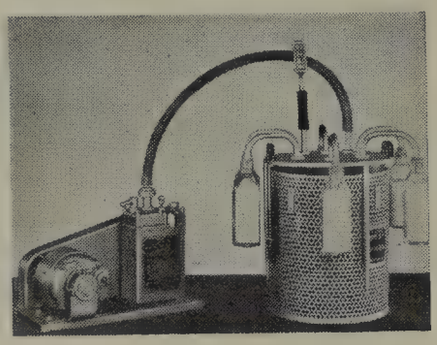

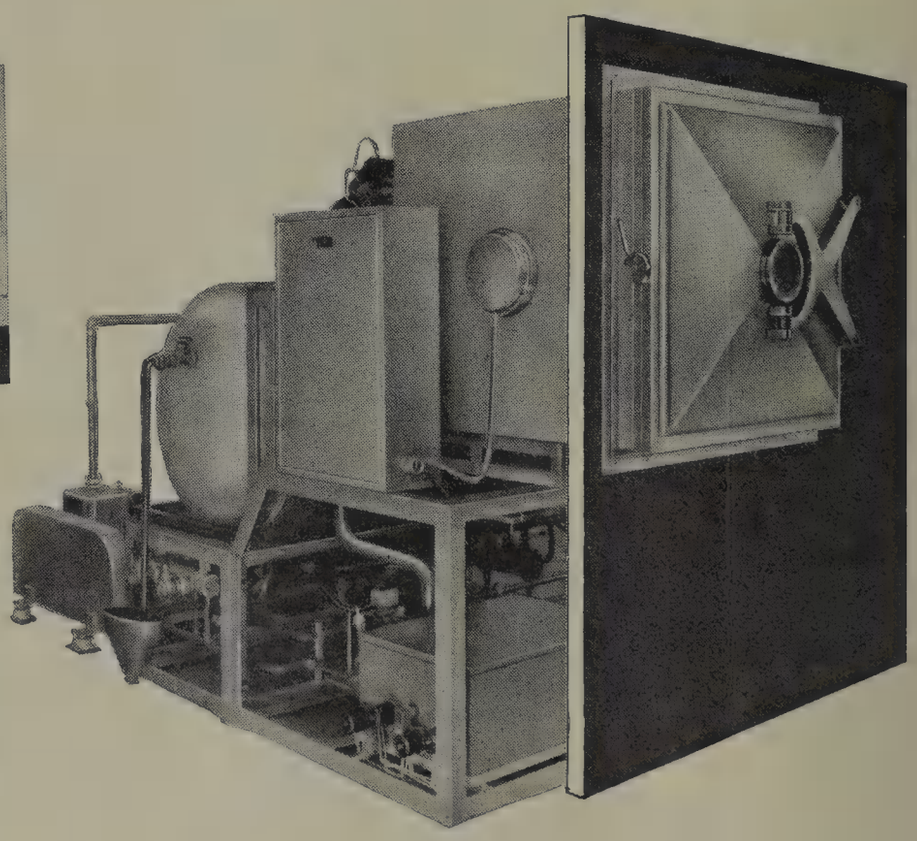

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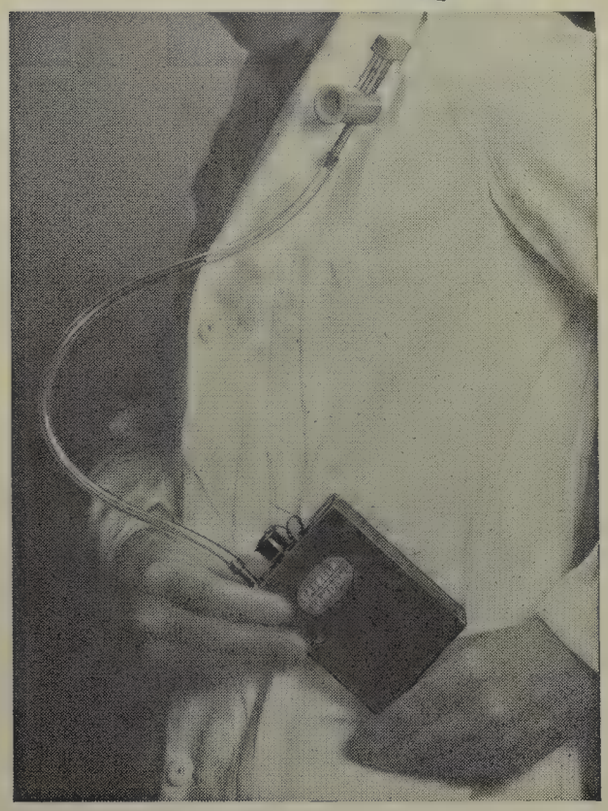

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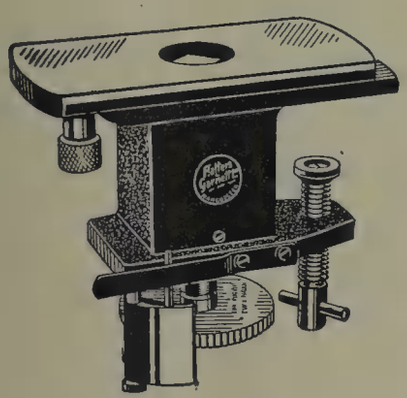

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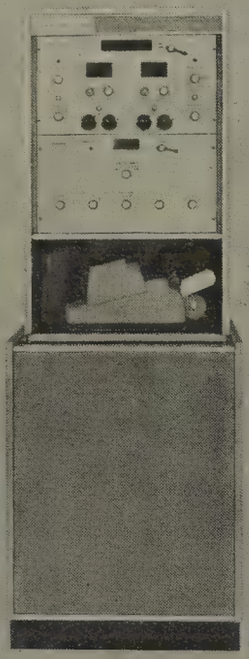

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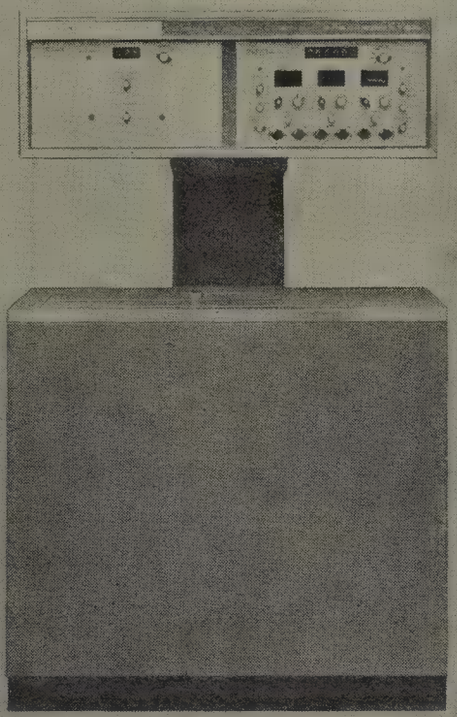

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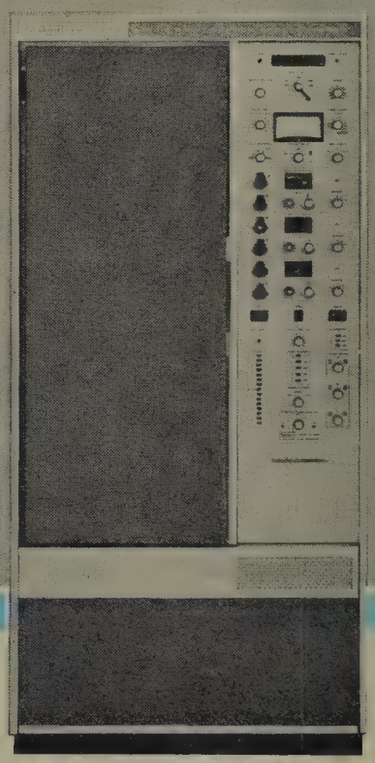

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IMPROVED ACCURACY IN THE ANALYSIS OF SOLIDS BY SPARK SOURCE MASS SPECTROMETRY

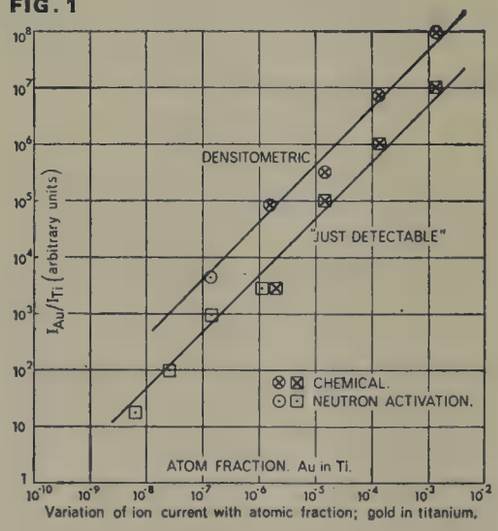
Since the possibility of using spark source ionization for the analysis of solids was first recognised ten years ago, the design of the double focusing mass spectrometer using Mattauch geometry has been considerably improved. And today one instrument—namely the AEI MS7—is capable of detecting impurities at levels as low as 1 part in 10^9. As a result the MS7—which, incidentally, was the first commercially available double focusing instrument to be built expressly for the analysis of solids—has found wide applications particularly where overall coverage of all elements and comparison analysis without standards are valuable.

Now the MS7 technique has been further improved by the careful control of certain parameters, and very good reproducibility and accuracy can be guaranteed. This bulletin reviews the parameters affecting analytical accuracy and outlines the methods of control developed by AEI engineers.

It has been shown that the sensitivity of the majority of elements differs from some standard such as iron by no more than a factor of 3. In other words, most relative sensitivity factors lie between 0.3 and 3. In the case of the MS7, the determination of relative sensitivity factors is considerably simplified by the fact that the response of the instrument is linear over a very large range of concentration. Indeed, Hannay & Ahearn[1] established linearity over the range 10^4 to 1 using doped silicon samples. More recently, W. A. Wolstenholme (AEI Consultant Lab.) has reported on the investigation of gold doped titanium samples covering a concentration range from 0.5% to .02 ppm by weight; a range of more than 10^5 to 1.

These samples were chosen because of their suitability for neutron activation and wet chemical analysis. Figure 1 shows the relative ion intensity of gold plotted against the concentration determined by chemical or neutron activation techniques. The "just detectable" line is an individual assessment for ion intensity and the "densitometric" line indicates that a microdensitometer was used to scan the spectral lines; the two graphs have been displaced for the sake of clarity. Matrix effects are generally very small, as is illustrated by the relative sensitivities for copper and steel standards reported in tables 1 and 2.

FIG. 1



Variation of ion current with atomic fraction; gold in titanium.

In copper and steel relative sensitivities are very similar for chromium (1·8 and 1·4) and for tin (1·3 and 1·1). Only for some low BP elements is there a marked dependence on the matrix, e.g. (1·4 and 2·6) for lead.

NOTE:

$$\text{Relative Sensitivity} = \frac{\text{uncorrected MS7 value}}{\text{known value}}$$

Homogeneity of standards and samples

Care has to be taken to use homogeneous standards or alternatively to increase the rate of consumption of sample above the usual 5 to 10 milligrams.

As it happens, however, the possibility of inadvertently using an inhomogeneous sample has been materially reduced by the introduction of more reliable methods of sample preparation.

TABLE 1

RELATIVE SENSITIVITIES AND REPRODUCIBILITY OF R.F. SPARK SOURCE ANALYSIS OF COPPER: JOHNSON MATTHEY CA2

30kV r.f.; 19.5kV accel. volts; pulse length and repetition rate varied in the analytical plates.

Impurity	Concentration given	p.p.m wt MS7	Relative sensitivity	% Standard deviation 8 separate analyses	% Standard deviation 15 repeat exposures
Bi	130	110	0.85	31	13
Pb	100	140	1.4	30	11
Sb	120	130	1.1	32	18
Sn	120	160	1.3	25	9.2
Ag	150	210	1.4	23	21
Ga	80	250	3.1	27	13
Cr	60	110	1.8	20	21

FOOTNOTE:

1 Hannay and Ahearn (1954) Anal. Cham 26 1956.

Reproducibility

The most important improvements in reproducibility have been achieved by careful control of certain instrumental parameters. As a result of recent investigations[2] it is now clear that the most important factor is one that is comparatively simple to control; namely, the ion accelerating voltage. When this is always set to the same value, and other source conditions are kept as constant as possible, reproducible analytical results are obtained. The principal reason is the improved constancy of the relative sensitivity factors for different elements. Table 2 shows how close agreement with known values is obtained when such relative sensitivity factors are measured and used to correct observed concentrations.

Variations in Photoplate

The standard deviations on identical exposures on a photoplate using a homogeneous aluminium standard indicate that the best standard deviation obtained for the different elements is about 10%. The standard deviation on isotope ratios, i.e. where the relative sensitivity factor is not involved and where a limited area of plate is used, show better figures of 3 to 4%. This indicates the likely variation due to the plate itself, and represents a limit that would remain even if a calibration spectrum of a standard was placed on the same photoplate as the sample to be analysed.

Reproducibility of Analyses

When different photoplates are used in separate analyses the standard deviation increases.

In repeat analyses different photoplates will be used and also source conditions may vary slightly. To cover the full range of exposures (10^7 to 1) it is sometimes necessary to vary the pulse length and repetition rate of the spark which might also contribute to the variations. An internal standard, although not essential, eliminates the need for such a wide range of exposures.

When the spark pulse-length and repetition rates for the analysis of a copper standard shown in Table 1 were held constant throughout the series of successive analyses of one of the standards, the minimum standard deviation did in fact decrease towards the minimum value previously obtained for repeat exposures (about 10%).

Accuracy of Analysis

The accuracy which can be attained with the MS7 when all parameters are properly controlled is perfectly exemplified by the data set forth in Table 2. In this case, of course, a standard of reasonable homogeneity has been used to establish relative sensitivity factors for the impurities in the given matrix.

It will be noted that only one standard is used, and that the levels of concentration in the standard do not correspond too closely to those in the unknown. A steel matrix has been taken as an example because studies of source conditions indicated that it seemed the most likely (compared with the aluminium and copper standards) to give poor results should the source conditions change. It is clear, however, that the results once corrected for relative sensitivity factors are in excellent agreement with those given by chemical analysis.

FOOTNOTE:

2 Halliday, Swift and Wolstenholme; Quantitative Analysis by Spark Source Mass Spectrometry, International Mass Spectrometry Conference, Paris 1964.

TABLE 2

ACCURACY OF ANALYSIS AFTER CALIBRATION: BUREAU OF ANALYSED STANDARDS LTD. MILD STEEL RESIDUAL SERIES SPECTROGRAPHIC STANDARD SS14

Pulse length 100 microsecs; 300 pulses/sec.; 30kV r.f.; 19.5kV accel. volts.
Concentration % wt

Impurity	MS7, average of eight analyses	MS7, corrected using SS12	Given Spectrographic value
Cr	0.26	0.18	0.185
Co	0.19	0.18	0.19
Ni	0.10	0.13	0.13
Cu	0.047	0.029	0.04
Zr	0.017	0.007	(0.005)
Nb	0.072	0.025	(0.05)
Mo	0.053	0.060	0.07
Sn	0.019	0.017	0.02
Pb	0.018	0.007	(0.0075)

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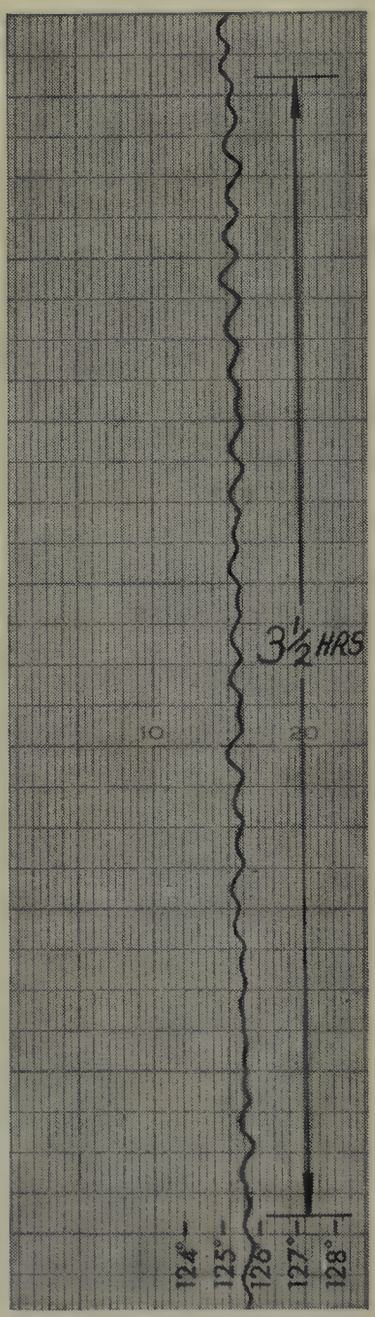

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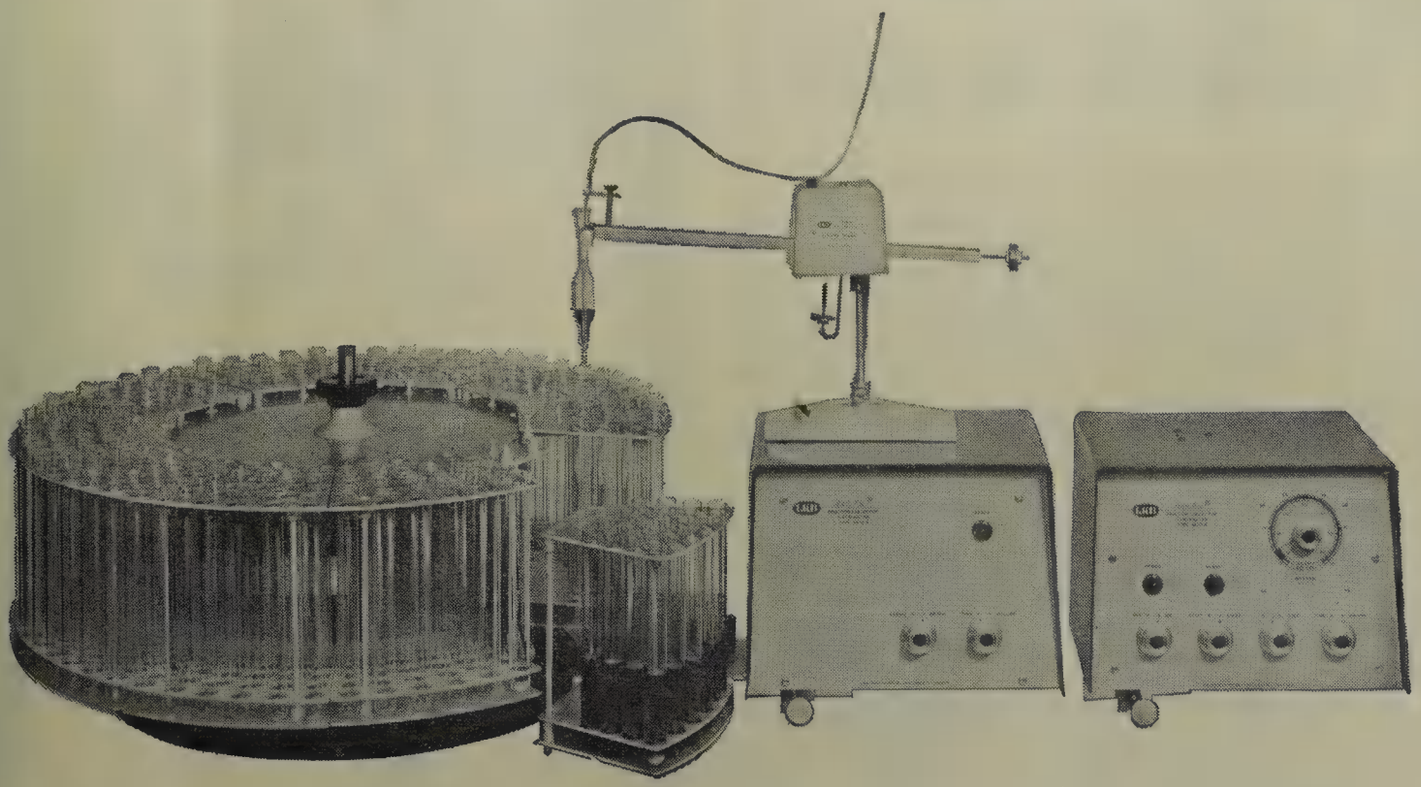

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(1) E. Gurr (1965), Rational Use of Dyes in Biology & General Staining Methods. (Leonard Hill (Books), London. Williams & Wilkins, Baltimore.)

(2) M. A. MacConaill & E. Gurr (1964), *Irish J. Med. Sc.* (June).

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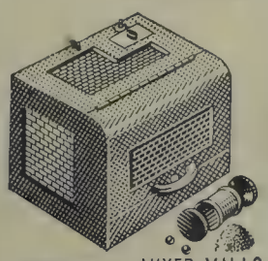

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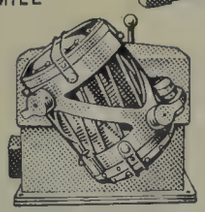

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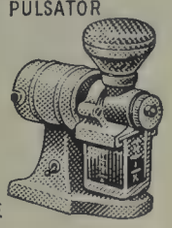

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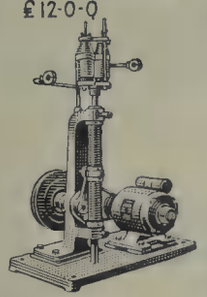

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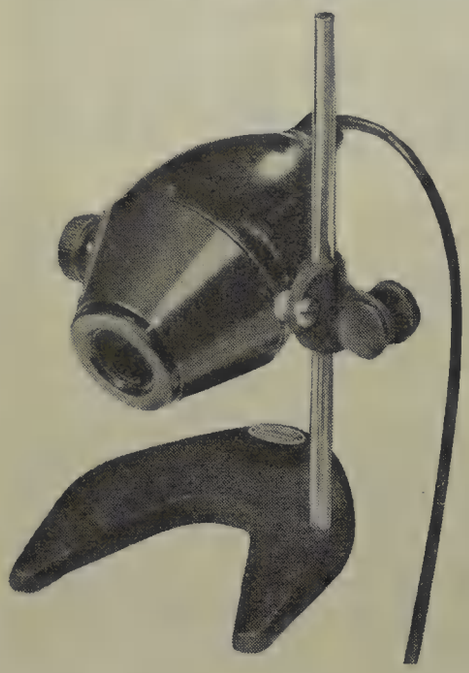

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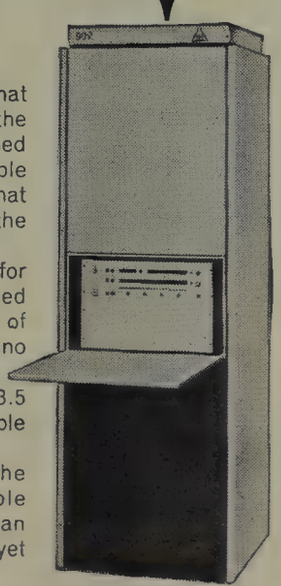

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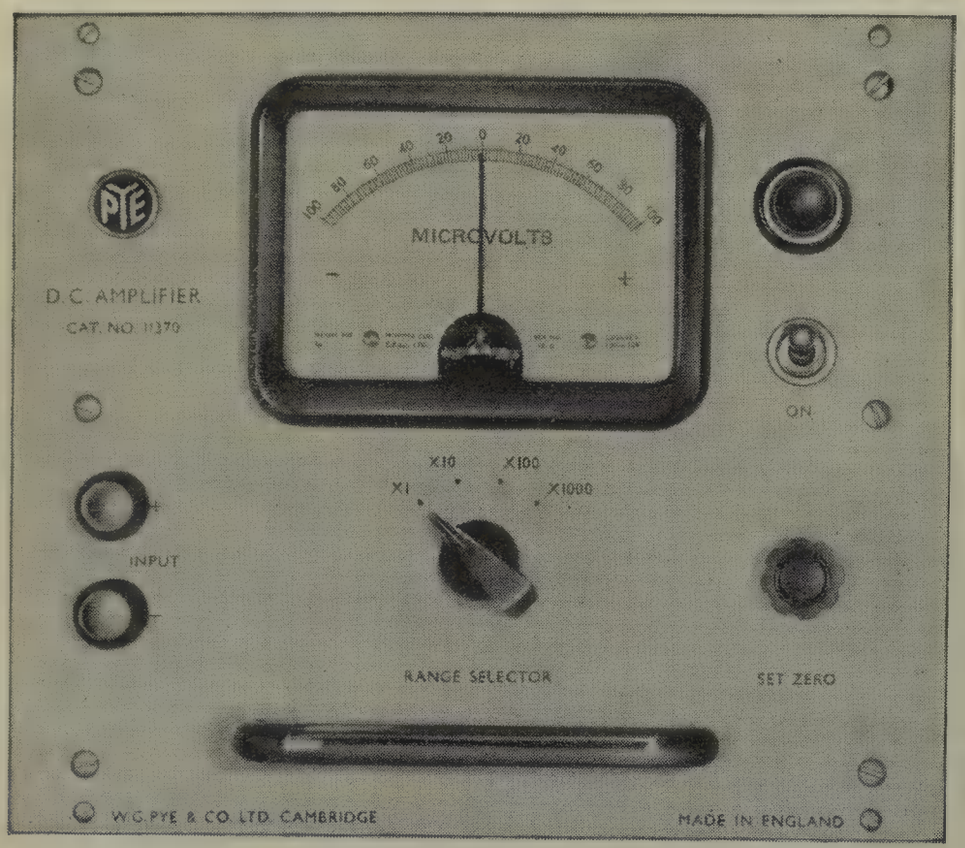

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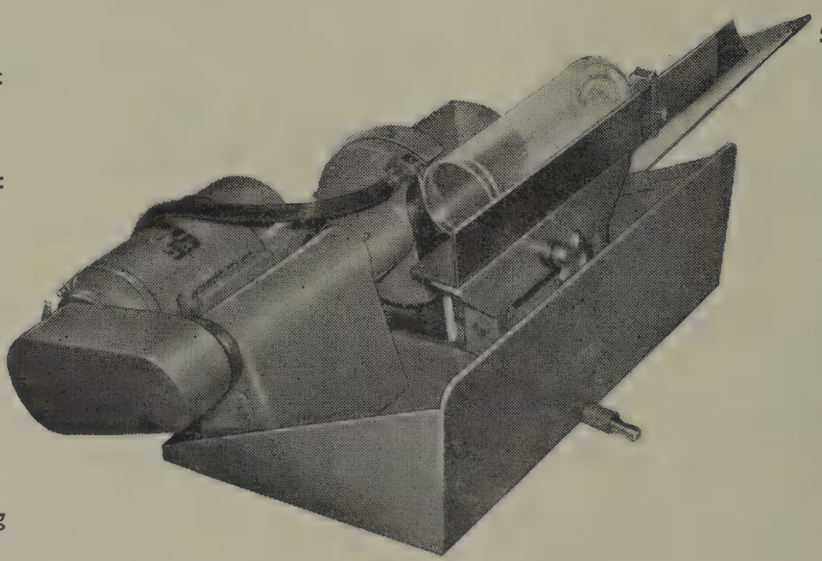

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INDUSTRIAL RESEARCH AND THE RESEARCH ASSOCIATIONS

LAST February, in his Jubilee Memorial Lecture to the Society of Chemical Industry on the organization of basic research for the British chemical industry, Prof. J. W. Mitchell made some passing reference to the research associations, as well as to sponsored research and to the research institutes as supplementing the research effort within industry itself. The last two, he suggested, had four important functions: first, they could bring firms together in a joint project of mutual interest, though possibly not of immediate commercial significance, and probably at a smaller investment than would be required if the firms undertook it individually. This is obviously something in the nature of a small and temporary research association.

Next, they can supplement the firm's own industrial research effort at a time of crisis by undertaking sponsored confidential work. This represents in effect a temporary expansion of the firm's own research laboratories. Thirdly, Prof. Mitchell envisages such institutes as undertaking unsponsored basic research to acquire new knowledge of value to the industry as a whole, this function representing rather that of a central laboratory and perhaps more particularly the type of basic research in which some research associations seek to engage. Fourthly, he places the educational function arising out of the opportunities provided for graduates to extend their experience in research.

Prof. Mitchell's comments on the critical size of a research laboratory, which have previously been discussed (*Nature*, 207, 113; 1965), are highly pertinent here, as they are also in relation to his much briefer remarks on the work of research associations. The inability of most of the smaller firms to provide the financial resources required to establish laboratories of at least this critical size is one of the fundamental reasons for establishing a research association. Moreover, contrary to the customary protestation of the smaller firm, it is to them rather than to the larger firms of the industry that the benefits of co-operative research mainly accrue. Prof. Mitchell does not believe that sufficient basic research is as yet being undertaken in Britain to meet the needs of the medium-sized and smaller firms. Instead, he suggests that the more successful research associations have worked to improve services provided essentially on a non-competitive basis and have also undertaken research on improving and characterizing well-established products and developing equipment for standardization and control. He recognizes that difficulties arise over the granting of rights for developing and manufacturing new products based on discoveries or inventions made by a research association, but he does not refer to the inherent advantage that the industrial research laboratory inside a firm necessarily possesses over one outside through the closer contact with the production side or the customers' needs.

About the same time as Prof. Mitchell's lecture was delivered, the Association of Scientific Workers submitted to the Ministry of Technology a memorandum on industrial research associations. In this memorandum the Association argues that research associations should be a principal means for putting ideas across to industry and for making a scientific and technological impact on the industries served by the associations. A research association, it suggests, would serve its industrial members best if it consistently looked to the future, and to a fairly long-term future. The associations should also accept a considerable responsibility for disseminating information, particularly to the smaller firms which are less likely to spend money and effort on an internal information service. This function is regarded as including the use by management of the information already available.

The memorandum admits that there are wide differences in the effectiveness with which different research associations fulfil their role, and these differences are attributed at least in part to the methods by which the research associations are financed—this affecting in turn the amount of money available. Recognizing also that industries which are lagging technically tend to lose interest in research and become even more backward, the memorandum suggests that amalgamation of appropriate research associations might give a firmer basis for development and that it would be worth while examining industries without co-operative research and co-ordinating the research efforts of companies undertaking substantial research in the same field. This latter somewhat naïve suggestion is not discussed further, but it is proposed that all research associations should operate within a national plan and be linked with the economic development council structure.

The present methods of finance are then criticized in some detail, especially for their failure to provide sufficient assurance of a reasonable level of income over a period of, say, five years: this is regarded as quite impossible if a research association has to depend on voluntary subscriptions. The position is made worse by the instability of prices and recurrent mergers and the like. The memorandum criticizes the progressive stiffening of grant terms by the Department of Scientific and Industrial Research, and it makes the valid point that too much effort has to be spent by directors of research in securing funds for research. Earmarked grants and civil research contracts are regarded as tending to increase financial strain and should not be looked on as a substitute for improved contributions from the Government to the regular programme.

As a consequence of this financial situation, the facilities for research in most associations compare poorly with those of Government stations. Total expenditure per qualified scientist and engineer in the research associations is put at much lower than the estimated average of £8,000 for all manufacturing industries in 1960; that for the research stations of the Department of Scientific and Industrial Research (now the Science Research Council) exceeds this figure. The consequent poor salary scales and prospects compared with the Scientific Civil Service make it difficult to attract and retain competent staff, and on the whole staff relations generally need improvement. The Association of Scientific Workers contends that an assured, adequate and independent income would immediately improve such relations. The unsatisfactory financial situation, of course, also hinders the acquisition of modern scientific equipment.

The system of finance is intimately linked with the method of control, and the memorandum maintains that the methods practised by the heads of businesses who dominate the Council of Research Associations are not always appropriate to the needs of technical departments. Moreover, financial and general control is largely a part-time interest of members of these Councils, leading to

haphazard and meretricious decisions, which may be based on narrow financial considerations. As a result, levels of expenditure may fall below the minimum necessary for efficiency and effectiveness, and voluntary levies agreed with the Board of Trade may not necessarily be fixed to ensure a sufficient level of expenditure. The memorandum also asserts that representations of the Department of Scientific and Industrial Research on the Councils and the Official Visitors are ineffective in influencing policy in proportion to the Government stake in the research associations.

Seven proposals are advanced in the memorandum to improve this position. First, a compulsory levy of 0·2 per cent of net output of the industries concerned is suggested in order to double the present total income of £10 million. Within this concept of a statutory levy, the Government contribution could be increased where necessary to pound for pound with the industrial contribution. Specifically, the appropriate Government agency should examine the need for establishing a research association, with a compulsory levy, for certain sections of the chemical industry. Possible amalgamations should be carefully considered and, in consultation with industry, the Minister of Technology should examine the programmes of all research associations. One function of the research associations should be to advise the Government in connexion with placing civil research and development contracts with industry. Establishment of an effective advisory service, using the most advanced techniques, is also recommended to ensure the dissemination of knowledge and techniques and their application to industry, and all research associations should participate in this service.

To some extent this last-mentioned recommendation anticipated the establishment of the Office of Scientific and Technological Information, but its effectiveness presupposes the existence within the small firm of those at the top capable of appreciating the significance of new knowledge and possessing the authority to take appropriate action. In most small-scale industry this condition is not fulfilled, and here, like so many other reports and memoranda, the memorandum is not sufficiently ruthless. There is too much disposition in Britain to protect the inefficient and it is this practice above all that must be ended. The research associations will never serve Britain effectively if they are used to feather bed the inefficient and backward.

Also early this year Dr. F. N. Woodward, scientific counsellor to the Organization for Economic Co-operation and Development, submitted to the Organization a survey of the different types of research association in member countries with particular reference to their organization, co-ordination, methods of operation and resultant benefit to the industries they serve. This report has now been published under the title *Structure of Industrial Research Associations**, but, although at some points the report supports some of the recommendations of the Association of Scientific Workers, there is such wide variation in the extent and character of Government participation from country to country that generalization is extremely difficult. Very little light is, in fact, thrown on the deeper aspects of co-operative research and the conditions in which it functions most successfully. By and large, the survey adds surprisingly little to the assessment of the research association movement in Britain contained in a series of articles in *Research* and since published as a supplement to Volume 15 in 1962, or even, for that matter, to Prof. R. S. Edwards's earlier book, *Co-operative Industrial Research*, or the report of the Federation of British Industries on *Industrial Research in Manufacturing Industry, 1959–60*.

The factual data in Dr. Woodward's report show that the industrial research association has developed to a much larger extent in the United Kingdom than elsewhere in Western Europe: the income in Britain of some £10 million in 1962 was more than double that in Germany, which comes next, though it is less than that expended in France on the rather different industrial research centres, which amounted to some £16·7 million in 1961. The idea, moreover, differs significantly in structure and working philosophy from country to country, and its development from the British prototype has been influenced to a marked degree by historical background and local requirements. None the less, the disadvantages and shortcomings of the research association scheme, as revealed by Dr. Woodward's enquiry, coincide with observations in the memorandum from the Association of Scientific Workers. This is notably so in the criticism of the excessive proportion of time which a director of research is required to spend on raising money, of the slender resources of most of the associations and their inherent disadvantage as against the industrial research laboratory of a private firm.

In addition, the research associations in general are regarded as not possessing a good public image either in the industries which they serve or in the scientific world from which they must attract their staff. This is unlikely to be assisted by the development of contract research. It is at this point that there is need for more fundamental and independent thinking about the precise functions of the research associations even in those fields in which co-operative effort, so far from being at a disadvantage compared with private enterprise, is the most economic as well as the most potentially fruitful method. It should be recognized that while a certain minimum scale of effort is essential, that scale should not be secured by the use of public money to support the inefficient or reluctant, and that there are circumstances in which a Government research station may be a more effective and prudent answer than an amalgamation of research associations or a solution involving the coercion of reluctant firms. There is warning enough of the dangers of precipitate action in Dr. Woodward's survey to suggest a cautious approach to some at least of the proposals of the Association of Scientific Workers. Certainly, the hard thinking and further enquiry necessary as to the merits of private or public enterprise should be kept free from any party political prejudice.

* *Structure of Industrial Research Associations*. By F. N. Woodward. Pp. 55. (Paris: Organization for Economic Co-operation and Development; London: H.M.S.O., 1965.)

CHEMISTRY OF THE SMALLER ORGANIC HETEROCYCLIC SYSTEMS

Heterocyclic Compounds with Three- and Four-membered Rings

Edited by Arnold Weissberger. Part 1: Pp. xi + 1–646. Part 2: Pp. xii + 647–1174. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 19, Parts 1 and 2.) (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 488*s*. the two parts.

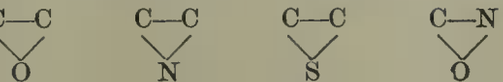
THESE two books, each separately bound and of considerable size, form Parts 1 and 2 of Volume 19 in the series *The Chemistry of Heterocyclic Compounds*

which, under the able editorship of Dr. A. Weissberger, have been appearing steadily and (in general) with increasing size since 1950. Some of the more recent volumes have appeared in two or more parts, and the number of actual books in the series is now twenty-five.

Part 1 deals with four types of three-membered ring systems and Part 2 with six types of four-membered systems: the pages of the two Parts are numbered as one volume, and Part 2 has an author and a subject index covering both Parts.

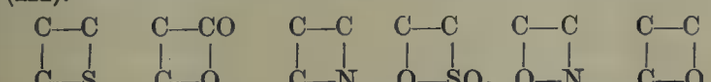
In view of the number of volumes which have preceded Volume 19, it might cause some surprise that these smaller and simpler systems have hitherto escaped attention in this series. Dr. Weissberger in the preface points out that heterocyclic chemistry has two closely related aspects, namely, the investigation of different derivatives of the respective cyclic systems, and that of the reactions in which the heterocyclic systems themselves undergo change. The latter aspect, he notes, is particularly important with heterocyclic systems having less than five atoms in the ring, some of which belong to the most reactive compounds in organic chemistry. This high reactivity undoubtedly underlies the recent wide extension of the study and the technical application of most of these compounds: it also probably explains the comparative slowness of earlier development, for less reactive and more stable systems attracted greater attention.

The contents of the two Parts can be briefly indicated. Part 1 contains chapters devoted individually to the systems listed as ethylene oxides (I); aziridines (II);



(I) (II) (III) (IV)

ethylene sulphides (III) and oxaziranes (IV). Part 2 contains the systems thietanes (V); β-lactones (VI), trimethyleneimines (VII), and a brief note on β-sultones (VIII*A*) and 1,2-oxazetine (VIII*B*), and finally oxetanes (IX).



(V) (VI) (VII) (VIII*A*) (VIII*B*) (IX)

An interesting point of nomenclature arises here. The International Union of Pure and Applied Chemistry (IUPAC) has recommended specific names for almost all heterocyclic systems up to the 10-membered units, and these names are now incorporated in the recent second edition of the *Ring Index* and its two supplementary volumes. Some of these names are far from self-explanatory and differ markedly from the older accepted names. It is interesting to note the extent to which they are accepted and used by the authors of Parts 1 and 2.

Dr. André Rosowsky entitles Chapter 1, on the compounds having the reduced system (I), "Ethylene Oxides", and points out that the IUPAC name is 'oxirane', and states: "in deference to common usage the present chapter will retain the terms 'apoxide' and 'ethylene oxide'". Dr. Fanta in Chapter 2 uses the systematic name 'aziridines' for compounds having the reduced system (II). Dr. Reynolds and Dr. Fields (Chapter 3) give the title "Ethylene Sulfides" to compounds having the system (III) and state: "The name thiirane is in accord with good nomenclature, but it has been slow to find a place in the literature". Dr. Emmons adopts the systematic name 'oxaziranes' throughout Chapter 4 for compounds having the system (IV). Dr. Etienne and his collaborators in Chapter 5 use the term 'thietane' for compounds having the reduced ring (V), but abandon it for the corresponding sulphonium salts (p. 650). Chapter 6 he entitles "β-Lactones", and states: "Nor does the (systematic) designation 2-oxetanone . . . seem to have been adopted". Dr. Moore gives the name "Trimethyleneimines" for Chapter 7 on compounds having the reduced system (VII), but in the text refers to them throughout as "azetidines". Systematic names are used for the remaining three systems. This summary will indicate the degree of confusion which still exists in the nomenclature in use for even simple heterocyclic systems.

These two books contain such a wealth of information that a true review, in the sense of a critical assessment of the merits and demerits of the work presented, cannot be briefly achieved. The general nature of the treatment of each class, and of the information presented, can best be shown by reference to Dr. Rosowsky's excellent exposition of the ethylene oxides in Chapter 1, which, running to 523 pages finishing with 2,040 references, is almost a book in itself. It contains the sections: (1) "Physical Properties" (that is, molecular geometry; energetics; infra-red, ultra-violet and nuclear magnetic spectroscopy; theoretical models); (2) "Occurrence of Epoxides in Nature"; (3) "Synthesis of Epoxides"; (4) "Chemical Reactions of Epoxides"; (5) "Analytical Methods"; (6) "References": sections (3) and (4) are presented in considerable detail. This chapter incidentally reveals the contribution made by the French chemists, from the early work of Wurtz to the later work of Tiffeneau and others.

The remaining chapters follow much the same pattern, and the complete volume forms a very thorough and highly valuable record of chemistry of these smaller ring systems.

It is noteworthy that the synthesis of oxaziranes (Chapter 4) was first achieved in 1956–57 by three different research groups, and that in Chapter 8 only one example of a 1,2-oxazetidine, prepared by Staudinger and Jelagen in 1911, can be recorded. Our knowledge of the chemistry of such systems must still be incomplete.

One might perhaps question the space devoted in Chapter 3 to the "Table of Physical Properties" (6½ pages), for only the boiling (or melting) points are recorded for certain compounds: similarly in Chapter 6, Table 2 (18 pages) and Table 3 (10 pages) giving the physical properties of aliphatic and aromatic β-lactones, respectively, also provide very limited information, and certain pages of the Table contain only three of the more complex derivatives. The sections in this Chapter on the preparation of β-lactones have been largely anticipated by the recent Volume 7, Part 2, of *Methoden der Organischen Chemie*, by Houben-Weyl (*Nature*, 204, 1122; 1964): the latter is, however, strictly limited to synthetic methods, whereas Chapter 6 of the present work gives a full treatment of the whole subject.

It should be mentioned that the preface concludes with an expression of deep gratitude from Dr. Etienne and his colleagues for the faultless translation of their Chapters 5 and 6 from the French by the late Mrs. Weissberger, to whose help in his life and work Dr. Weissberger pays an eloquent and moving tribute.

F. G. Mann

ANALYTICAL CHEMISTRY

Organic Complexing Reagents

Structure, Behavior, and Application to Inorganic Analysis. By D. D. Perrin. (Chemical Analysis: a Series of Monographs on Analytical Chemistry and Its Applications, Vol. 18.) Pp. xi+365. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 90*s*.

THE doyen of analytical chemistry, Fritz Feigl, has remarked that the story of modern analytical chemistry is the story of co-ordination chemistry. This remark has made a profound impression on many a young research worker, as has S. E. Q. Ashley's more recent comment that the chemistry is going out of analytical chemistry. These observations apparently point in two directions, but in truth they do not really conflict. While

an ever-increasing proportion of analyses is being carried out instrumentally rather than by balance, beaker and burette, the instrumental hardware, except in a few cases such as mass spectrometry, relies on chemical reactions. These reactions are largely based on complexation and are usually further backed up by refined modern separation techniques which again rely almost exclusively on complexation. The comments may, therefore, be regarded as complementary.

Complex formation between aquo-metal ion complexes and neutral or negatively charged ligands extensively modifies the normal (aqueous) chemical reactions of most ions and many characteristic physical properties usually associated with them. Thus many metal ions become central in anionic complexes and hence respond differently to sorption processes such as ion exchange and ion association reactions. Many become bound in uncharged complexes and precipitate from solution or can be persuaded to partition preferentially into water immiscible solvents. Some even become capable of being volatilized or distilled. In most cases changes are brought about in absorption characteristics in one or more regions of the electromagnetic spectrum. Not infrequently unusual valency states are preferentially stabilized by complexation, and the changes in oxidation potential which accompany such reactions open out avenues of examination by electrochemical techniques. Complex formation even extends a profound influence on to flame plasma techniques such as atomic absorption spectroscopy and flame spectrophotometry. In these cases, the supply of cations bound as neutral chelate or ion-associated complexes and dissolved in organic solvents can give considerable enhancement of sensitivity by purely mechanical surface tension/viscosity effects and can produce new reactions due to shifts from (normal) endothermic to exothermic dissociation–decomposition mechanisms. Many interionic interferences in flame techniques are also simply eliminated by virtue of complex formation. Similarly many apparently intractable techniques can be profoundly indebted to complex formation for their success. Thus, radioactivation analysis relies heavily on the solvent extraction of chelate complexes from aqueous solution to achieve the separation-concentration processes on which the radiocounting procedures depend.

Most modern texts on analytical chemistry are, of course, concerned with instrumental analysis and are at pains to emphasize the basic physical chemistry, the physics and even the electronics underlying these procedures. Few of them pay much attention to the chemistry involved. *Organic Complexing Reagents* is concerned with just this facet and takes the same pains to set the scene behind the chemical reactions in terms of modern theories of inorganic chemistry. In my opinion it succeeds admirably in this and the result is stimulating and worthwhile. The only book which has previously been written on this topic is Feigl's *Specific, Selective and Sensitive Reactions*, and although the latter is now sixteen years old, it is still one of the most fascinating and important of all books on analytical chemistry. This new book is written from the same point of view of basic chemical reactivity and is the first real development since the original publication. Unquestionably this is an important book of unusual interest and should be found by most analytical chemists and by many others to be a sparkling jewel of chemical interest set against the grey background of instrumental methodology on the bookshelf. It is equally certain that it will bid fair to become one of the most thumbed volumes on the shelf because of the wide-ranging nature of its interests.

The text itself is well arranged. A short chapter on chemical bonding set in terms of valence bond, molecular orbital and ligand-field theory follows a brief introductory survey of the phenomena of complex formation between metal ions and organic ligands, and sets the scene for subsequent chapters. This treatment is concise and entirely adequate for those who have had some experience of modern theories, but those who are not familiar with them may well have to supplement their knowledge by further reading from the recommended list of books in order to make full use of the discussions which follow in subsequent chapters. The stereochemical configuration and stability of metal complexes are considered in terms of polarizability and covalent character and the thermodynamic nature of the formation reaction, etc. Numerous examples of ligand-field effects are cited in the transition series. The effects of complexation on oxidation potential are discussed in relation to factors which determine the magnitude of these potentials, ligand-field stabilization energies and so on, with examples drawn chiefly from among iron and copper complexes and cyanide complexes in the transition series.

A résumé of factors which govern rates of formation and dissociation introduces a chapter of a more applied nature on the effects of complexation on the concentration of cations in aqueous media and which is, of course, principally concerned with complexiметric titration, with masking and with demasking. Surface phenomena associated with complex formation are related to analytical techniques such as ion-exchange, chromatography, use of adsorption indicators and, somewhat illogically in this context, polarography. The chapter on visible and ultraviolet absorption spectra includes some useful notes on types of spectra and considers re-emission phenomena in relation to fluorescence and chemiluminescence. It also incorporates an interesting survey of the design of ligands in relation to absorption spectrophotometry. The two chapters on the solubility of complexes and extraction into organic solvents give an excellent coverage of their subject-matter and relate gravimetric and separation procedures to the nature of the reagent reacting with the metal cation. The reaction of inorganic anions with organic reagents and with metal complexes is considered from a variety of angles in a short but thought-provoking section. The final three chapters deal with procedures for detecting and determining inorganic neutral molecules, the general analytical properties of the elements and with some speculative matter on seeking new organic reagents for use in inorganic analysis. The book ends with a full subject index; there is no author index.

This, to my mind, is one of the most important books to have been written on inorganic analytical chemistry within recent years.

T. S. West

SOLID-STATE PHYSICS

Solid State Physics

Advances in Research and Applications, Vol. 15. Edited by Frederick Seitz and David Turnbull. Pp. xvi + 505. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1963.) 118*s*.

THE series of volumes entitled *Solid State Physics* has come to be of considerable importance to solid-state physicists. For most people active in this field there are several articles, scattered through the volumes, to which they and their research students return again and again. The articles are, for the most part, closely written and demand quite intensive study if they are to yield a substantial portion of their content. Most will offer something to the generally informed physicist who reads quickly through them, but this is not their main function. One wonders in some cases whether this class of reader, and even the experts and prospective experts (not to mention reviewers), might be helped by a somewhat more emphatic distinction between the main points to be made in the article and subsidiary elaboration. The articles fall little short of small books, and a suitably discreet modification of the 'on a first reading' formula might not be inappropriate.

Volume 15 of the series resembles previous ones in being occupied mainly by essentially theoretical articles in which the properties of well-defined models are worked out and compared, more or less thoroughly, with experiment. The longest article, that by R. W. James on the "Dynamical Theory of X-Ray Diffraction", falls very clearly into this category. It will prove of great importance to workers in electron diffraction as well as to those concerned with X-rays. In treating the "Elementary Theory of the Optical Properties of Solids", F. Stern combines a discussion of phenomenological electromagnetic properties, including the wave-length dependent dielectric constant, with treatments of the free electron gas, electrons and holes in semiconductors and optical modes in ionic crystals: the application of dispersion relations is considered. L. J. Sham and J. M. Ziman discuss carefully the basic concepts of "Electron-Phonon Interaction" and the resulting electron scattering in solids. The considerations which they present resolve a number of questions and doubts on some fundamental points, and lead to clear definitions of the matrix elements which appear in the theory; topics treated include deformation potential, pseudopotential and screening effects. In discussing "Spin Temperature and Nuclear Relaxation in Solids", L. C. Hebel impresses one further with the rich interplay of theoretical and experimental activity (theorist and experimenter often being the same person), which has characterized work in nuclear magnetic resonance.

The first article in the volume, that by G. Borelius on "Changes in Energy Content, Volume, and Resistivity with Temperature in Simple Solids and Liquids", is in a different category. Here the experimental results dominate, and such theoretical discussion as there is frequently emphasizes the apparent inadequacies of present-day theory. It is good to see this approach represented.

C. W. McCombie

SCHRÖDINGER'S LOGIC

My View of the World

By Erwin Schrödinger. Translated from the German by Cecily Hastings. Pp. viii+110. (Cambridge: At the University Press, 1964.) 18*s*. net; 3.50 dollars.

THIS book consists of two long essays, hitherto unpublished. Although separated by a long period—the first was published in 1925, and the second in 1960—there are close links between them in respect of subject-matter and treatment. There are also affinities with the reflexions with which Schrödinger concluded his book *What is Life?* (1944), although this book is not mentioned in the present work.

In these pages Schrödinger puts forward a philosophical and indeed metaphysical view of the world, of human experience and of human nature. He rightly does not attempt to derive these conclusions from theories in the natural sciences but adopts a starting point which is philosophical—that is to say, one in which the sciences themselves are located in the wider context of human experience of the world and of moral relationships. Schrödinger accepts the validity of Kant's criticism of speculative metaphysics but argues, in my view correctly, that to some degree metaphysics, in the sense of presuppositions of enquiry, is indispensable. The particular problem which concerns him is the basic one of the relation between experience and reality. He points out that the commonsense view that independent observers, or 'selves', have similar experiences of the same external world is not self-authenticating; here Schrödinger is clearly under the influence of his mentors, Mach and Avenarius. There is no doubt that Schrödinger is right in saying that this is a metaphysical point of view in the sense explained. It is with less plausibility that he seeks to supplant it with an alternative and indeed inverted metaphysical doctrine which he derives from the Vedanta. Instead of thinking of different selves experiencing a common world, he invites us to think of a multiplicity of selves sharing a common consciousness. According to this idea there is no ultimate distinction between different minds or indeed between consciousness and its object. To a Westerner, at any event, this is a paradoxical view which lacks inherent plausibility; unfortunately Schrödinger does not offer convincing arguments in its support.

Another difficulty is that Schrödinger appears to think that it goes some way to solving a problem the genuineness of which is very much open to doubt. "If she who is now your mother had cohabited with someone else and had a son by him, and your father had done likewise, would you have come to be? Or were you living in them, and in your father's father . . . thousands of years ago? And even if this is so, why are you not your brother, why is your brother not you, why are you not one of your distant cousins?" To ponder why I am not my brother, or where or what I would be if my parents had not met, is to induce a kind of intellectual vertigo for which the cure is not the Vedanta, but rather a closer attention to the logic of our language. The reason why I am I and not someone else, or that this pencil is this pencil and not another pencil, is to be found in the logical principle of identity—that is, a tautology. Schrödinger is not the first, and will not be the last, to find in logic a source of wonderment, and in metaphysics an apparent resolution of it—but what precisely is the 'problem', as distinct from the wondering?

C. K. Grant

SPECTROSCOPIST'S VADE-MECUM

Flame Spectroscopy

By Radu Mavrodineanu and Henri Boiteux. (Wiley Series in Pure and Applied Spectroscopy.) Pp. xiv+721. (New York and London: John Wiley and Sons, Inc., 1965.) 315*s*.

FLAME Spectroscopy, by Drs. R. Mavrodineanu and H. Boiteux, is probably one of the most detailed and comprehensive works on flame emission spectroscopy and atomic absorption spectrophotometry which has ever been written. It may be regarded as a modernized edition of the original *L'Analyse Spectrale Quantitative par la Flamme*, published in France in 1954.

The authors deliberately avoid the discussion of practical laboratory methods for the analysis of specific elements or compounds, although most extensive references are given to published papers of this type.

The present publication has a far more generic approach to the theoretical and instrumental aspects of flame spectroscopy, the descriptive text being embellished with photographic illustrations and diagrammatic representations of the highest quality and most commendable clarity. The coverage given to spectrographic instrumentation is truly international, the products of the world's most well-known instrument makers being given detailed attention. The construction, performance and general physical parameters of these spectroscopes, etc., are concisely listed for rapid comparative reference.

The present work is presented in three parts comprising twenty-two chapters. The first part deals in a most exhaustive fashion with the fundamental principles of flame production; inflammability, burning velocity, temperature contours, etc., are but a few of the topics lucidly described. In addition to the less common flames of cyanogen-oxygen or hydrogen-perchloryl fluoride, much attention is directed to plasma jet, atomic hydrogen and high-frequency flame systems.

Numerous types of burners and atomizers are clearly described, the range covered extending from the apparatus of Beckman (1899) and Lundegårdh (1936) to the ultrasonic device of Peskin and Raco (1963). Much valuable detail is also given regarding the properties of materials

suitable for the construction of these flame spectroscopic accessories.

Sensing elements of the photographic plate and photosensitive type receive detailed treatment. Interfering phenomena are listed; the advantages of the absorption method for resonance radiation investigations is noted and many allied topics of vital spectroscopic interest are clearly presented. Part 2 deals with the fundamental theoretical treatment of atomic and molecular spectroscopy; modern ideas of atomic structure are related to values of emission frequencies. Parity, selection rules, coupling, isoelectronic sequences and hyperfine structure are just some of the numerous topics discussed. The band spectra of molecules receive similar detailed treatment.

Subsequent chapters contain extensive data from spectral examinations of atoms and molecules excited in acetylene flames. The wave-length tables are accompanied by photographic reproductions of typical spectra and provide a reference work of the greatest value and utility to the practising analyst.

The publication may appear expensive (315*s*.) but it is a massive, well-produced work of the greatest value and authority. It will provide a powerful teaching and experimental aid and contains much that is new and scientifically helpful. Since it must prove a well-thumbed vade-mecum, the publishers have thoughtfully provided a durable additional cover-case which will help to protect it from the buffetings of constant use. D. T. Lewis

THE CHROMAFFIN CELL

The Natural History of the Chromaffin Cell

By Prof. Rex E. Coupland. Pp. xi+279. (London: Longmans, Green and Co., Ltd., 1965.) 50*s*. net.

EVER since Vulpian in 1856 noticed that sections or extracts of the adrenal medulla could produce colours on oxidation, the significance of chromaffin tissue and the adrenal medulla in particular has interested a great number of investigators. So important did the adrenal medulla appear to be that for a time the vital functions of the adrenal cortex were completely ignored. Interest appears to have declined in recent years due to the counter-attractions of the postganglionic sympathetic nerve-endings.

The Natural History of the Chromaffin Cell has appeared at a most opportune moment to describe what has already been achieved and to point out the possibilities for further useful work on the study of the chromaffin cell. Until now it has been very difficult to obtain an overall picture of the published work on this subject since so many different specialists have been involved. Prof. Coupland has succeeded in combining the anatomical, histological and physiological data in a way that makes this book a fine reference source and at the same time a pleasure to read. He restricts his scope to a consideration of the chromaffin cell defined as "an element developed from the neuro-ectoderm, innervated by pre-ganglionic sympathetic nerve fibres capable of synthesizing and secreting catecholamines and storing them in sufficient quantities to give a positive chromaffin reaction with aqueous solutions of potassium dichromate or chromate". This, of course, excludes the enterochromaffin cells, mast cells and dopamine-storing cells. Without these a coherent account is possible. To include them would only confuse the story.

The first chapter is concerned with the histology, histochemistry and ultrastructure in which the various methods of preparation and staining are critically evaluated. Emphasis is placed on the histochemical methods available for the visualization of the catecholamines, but staining techniques for other cell constituents are also included, together with a section on the electron microscopy of chromaffin tissue. The embryological and foetal development is then described in which the evidence bearing on the origin of the chromaffin cells from the neuroectoderm is explored, including the recent evidence that some of the cells may arise from the neural tube. The chapter on the post-natal fate of the extra-medullary chromaffin tissue gives Prof. Coupland a chance to point out the problems associated with the use of human material and also to describe his own work in some detail. He also fully reviews the literature available on the innervation and blood supply to chromaffin tissue. In the chapter on the synthesis, storage and secretion of catecholamines, a fair balance is maintained between the apparently conflicting evidence concerning the actions of reserpine on the innervated and denervated adrenal gland. A whole book could be written about the metabolism of catecholamines; the author does well to cover the subject within a reasonable space without the chapter degenerating into a list of references. The chapter concerned with functions of chromaffin cells in the body also, surprisingly, contains a section on the metabolic fate of the released catecholamines. There is a brief survey of the occurrence and characteristics of tumours of chromaffin tissue. The book ends with a chapter on the comparative anatomy of chromaffin tissue throughout the many orders of vertebrates and invertebrates. These final two chapters must be included for the book to be complete and will serve to widen the knowledge of many.

At the end of each chapter there is a comprehensive list of references with titles. This removes the need for the numbering of references and cross-indexing, but an author index would have been useful. The subject index is adequate for a book of this size. There is the usual quota of minor misprints which detract little from the overall quality of the book, and even *N*-methylnoradrenaline is readily translated as *N*-methyladrenaline by a quick look at the list of references.

Prof. Coupland is to be congratulated on the excellence of this monograph and the publishers for the quality of production at, for these times, a reasonable price.

B. A. Callingham

TECHNIQUES IN CELL METABOLISM RESEARCH

Methods in Cell Physiology

Vol. 1. Edited by David M. Prescott. Pp. xiii+465. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 118*s*.

ALTHOUGH much of the progress and orientation of modern biochemical research on cell metabolism and its control has derived from investigations on bacteria, there are many problems relating to the regulation of cellular processes for the study of which larger unicellular organisms are more suitable. Such are the control of mitosis, nucleolar function, nuclear–cytoplasmic relationships, and pinocytosis, as well as problems relating to the control of special forms of differentiation, such as encystment. When cells are large enough to permit microsurgery, such as the transplantation of nuclei, or when synchronous mitosis or synchronous encystment can be induced, the opportunities for investigating cell regulation at a genetic or a biochemical level are enormously improved. Although various organisms with these properties are known, and have been extensively studied in the laboratory, many research workers who might feel inclined to use them for their own purposes, but are not already familiar with them, are liable to be put off trying to do so because the techniques for handling such organisms are unfamiliar and their difficulty hard to evaluate. With Volume 1 of *Methods in Cell Physiology* in their hands such doubts and hesitations should vanish.

This excellent volume contains a series of chapters devoted to the techniques for growing and handling cells

of different kinds, with discussion of their advantages and limitations for particular purposes. Each chapter is written from extensive practical experience, and gives details not only of how to grow these cells but also, where necessary, of how to stain them and how to make the special tools for handling them, such as those required for microsurgery. Cells suitable for synchronous culture include *Chlorella*, *Acetabularia*, *Euglena*, *Tetrahymena*, and mitotic synchrony can be induced in *Physarum polycephalum* by coalescence of the microplasmodia. Cells suitable for microsurgery of various kinds are *Stentor*, *Amoeba* and *Euplotes*, and grasshopper neuroblasts. A separate chapter is devoted to methods for studying pinocytosis, especially in amoebae.

There are also five useful chapters on autoradiography which alone would make this volume worth purchasing. These also are full of practical details for obtaining the best results in quantitative autoradiography, high-resolution autoradiography, and autoradiography of water-soluble materials and of mammalian chromosomes. A most valuable feature is the discussion of the theoretical and the practical limitations of the techniques advocated. Finally—and appropriately—J. E. Edström contributes a chapter on micro-extraction and micro-electrophoresis for determining and analysing nucleic acids in isolated cells which makes even this *tour de force* seem possible for other mortals. The editor, D. M. Prescott, is to be congratulated on the production of a thoroughly useful volume.

J. H. Humphrey

SURGERY AND SCIENCE

The Surgeon's Craft

By Prof. Hedley Atkins. Pp. x+201. (Manchester: Manchester University Press, 1965.) 15*s*. net.

THIS readable little book traces the development of the surgeon's craft from prehistoric times to the present day and is presented in a form readily understandable by the non-medical reader. It should therefore appeal to scientists engaged in the physical, chemical and biological sciences on which surgery has so extensively drawn to make many of the achievements described possible. Here in short compass is recorded how surgeons operate on the heart and other vital organs with impunity; that these achievements are of comparatively recent origin is shown by the startling statement that well into the nineteenth century surgery was "bloody, brutal and dangerous"! Asepsis and anaesthesia were soon to alter that fearsome state of affairs. The distinction which the author draws between the surgeon and his more intellectually inclined physician colleague is probably fair, but he makes it clear that, especially in university departments of surgery and in the Royal Colleges, more and more younger men are undertaking basic research, both biochemical and biological, and thus bridging the gap between the laboratory and the wards and operating theatre.

The thorough, exacting and protracted training of the surgeon, and what kind of man he is, are described. This should give the non-medical scientist the chance of comparing this with his own training and attributes. Culmination for the surgeon is reached on appointment to a consultant post, usually in his late thirties, and no doubt the question as to why the surgical training takes so long will be asked. The answer would seem to be the immense responsibility in dealing with human patients, and this is reflected in the financial awards which will be of great interest to other scientists.

The author sees a glowing future for surgery, despite the ultimate object of surgery being the abolition of surgery. By the combined attack of many scientific disciplines it is to be hoped that a disease such as cancer will one day be conquered and so make the mutilating operations needed for its complete extirpation a thing of the past. Truly then would the ultimate object of surgery be achieved. It is interesting to speculate what would be the effect on the surgeon's craft of the elimination of cancer, rheumatoid arthritis and cardiac and renal diseases. The solution of the homograft problem in transplantation would undoubtedly bring other outstanding problems into the surgical arena, and future advances must of necessity bring a profound change of outlook and approach for the surgeon.

Thus although *The Surgeon's Craft* still presents surgery in the main as a 'craft', the reliance of modern surgery on many scientific disciplines is obvious and should be a pointer to the training and occupation of the surgeon of the future. Prof. Atkins skilfully blends the clinical aspects of surgery with its scientific foundations. The proceeds of the sale of this book have been generously donated for the preservation of Down House as a memorial to Charles Darwin, from whom modern surgery drew much inspiration.

N. W. Nisbet

PRINCIPLES APPLIED

Genetical Principles and Plant Breeding

By Prof. Watkin Williams. (Botanical Monographs, Vol. 5.) Pp. x+504. (Oxford: Blackwell Scientific Publications, 1964.) 70*s*.

THE title of this book suggests a similarity of content with several others written on the theme of plant breeding in recent years, but it differs from them in at least two important respects. First, it is directed towards a student audience, principally to those who are studying in the general sphere of agriculture, and secondly, it consistently relates the pure and applied aspects of genetics without burdening the text with either crop botany or the intricacies of the routine methods of crop improvement. It is tailor-made for course requirements and aims to provide an understanding of the genetical principles governing the lives of flowering plants, and of the methods which lead to their improvement.

The first five chapters deal with such basic considerations as the genetic components, the organization of the genes, the chromosome complement, mutation, and the role of the cytoplasm in heredity. Basic Mendelism and descriptions of mitosis and meiosis are not included as a knowledge of these is presumed. Genetic systems governing sex determination and breeding behaviour occupy three chapters with especial emphasis given to incompatibility systems. The following two chapters are concerned with plant populations, their adaptation to their environment, and the influence of selection. The genetic basis of disease resistance forms the subject of the penultimate chapter and, finally, there is a description of special techniques such as chromosome substitution and addition, the utilization of haploids and the production of polyploids. The text is liberally supplied with line drawings, tables and photographs and the bibliography is adequate.

Genetical Principles and Plant Breeding is attractively written with a degree of lucidity which will make it popular with many. It covers the broad subject adequately and with the authority which we may expect from Prof. Williams's wide experience of practical plant breeding and genetics. It is refreshing to see attention directed to chimeras and plastid mutation though equally it is disappointing that *Xenia* receives no mention.

Although this book seems remarkably free from factual errors, like others which have gone before, it perpetuates a few fallacies which are now in danger of becoming regarded as facts. For example, emphasis is placed on the incomplete meiotic pairing of autopolyploids with its detrimental effect on fertility, and the relative natural rarity of such plants is attributed to these causes. What is here overlooked is the complete pairing and high fertility of many autopolyploids and the ready response to selection, either natural or artificial, of those which are irregu-

lar. To then presume that infertility is the explanation for the lack of success of autopolyploids is to ignore the fact that many allopolyploids have also to overcome, and do, a barrier of sterility even more extreme than that of autos, albeit at the diploid level.

The success of this book will ultimately be judged by generations of students, but already it seems likely to be assured. The contribution which it makes can scarcely be said to startle or surprise, rather will it interest and satisfy, both the students for whom it is intended and others of more advanced levels of learning. K. Jones

VASCULAR PLANTS OF EUROPE

Flora Europaea

Edited by T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters and D. A. Webb, with the assistance of P. W. Ball and A. O. Chater. Vol. 1: Lycopodiaceae to Plantanaceae. Pp. xxxii+464+5 maps. (Cambridge: At the University Press, 1964.) 84*s*.

THE publication of this volume (the first of four) means a most remarkable event to all biologists. To the layman and perhaps also to numerous biologists it may be a great surprise that no such work existed earlier. It is true that at the beginning of scientific botanical taxonomy the number of known species was so small that it was possible to publish works enumerating and describing them all. In his *Species Plantarum* (first ed., 1753), Linnaeus listed about 6,000 species in all, about 5,400 phanerogams and about 600 cryptogams. He believed the flora (and the fauna) of the tropics to be rather uniform and estimated the total number of plant species (of all groups) to be about 10,000. How wrong he was! The number grew rapidly and steadily through expeditions to various parts of the world. The microscope opened new worlds among the cryptogams. It became impossible to embrace the whole world in one Flora, and also other kinds of specialization became necessary. Besides phanerogamists (and pteridologists) there came bryologists, phycologists, mycologists and lichenologists. It has nowadays almost become a convention that a Flora without any specifying attribute means one treating only phanerogams or vascular plants (that is, phanerogams + pteridophytes). The *Flora Europaea* is no exception, for it is no complete Flora but one restricted to vascular plants.

Even the relatively poor phanerogamic flora of Europe exceeds numerically the 10,000 species which Linnaeus thought to represent the final and total sum of all plant species. The total number of known species of phanerogams alone is to-day judged to approach 250,000. Their final number cannot be estimated with any certainty as yet. In recent years the number of new species proposed seems to be on the decline, which may indicate that the stock of unknown species is now becoming exhausted. As to the European phanerogamic flora there remain certainly rather few species to be detected as new to science (or as new to Europe).

Why have we not had a comprehensive Flora of European vascular plants much earlier? The main reason is that floristic (and faunistic) studies have in the past mostly been restricted geographically by political or even provincial boundaries. A positive merit of such restricted areas is that the making of inventories can be more thorough and lead to relative completeness within reasonable time. But there are also negative merits. The limits of distribution of plants (and animals) do not follow administrative boundaries. Even where there has been no 'iron curtain' scientific contacts and field studies across the boundaries have been much too scarce, and so it may happen that the same plant is identified differently in different countries and, vice versa, that different species pass under the same name in different countries. Moreover, the full amplitude of intraspecific variation and the sterility barriers of a species cannot be elucidated without following it throughout its whole area.

Most European countries possess Floras of their own, often modern and good ones. There are also Floras of a more regional character, for example, those treating two or more of the Nordic countries. The most pretentious *Synopsis der Mitteleuropäischen Flora*, started in 1896 by Ascherson and Graebner, has remained an unfinished work, but Hegi's magnificent *Illustrierte Flora von Mitteleuropa*, begun in 1908, runs now into its second edition. The eastern part of Europe is included in the gigantic *Flora URSS* (30 volumes, 1934–64).

In the 1940's the German W. Rothmaler projected a *Flora Europaea*, which was to cover not only Europe but also the Caucasus, Transjordan and the parts of North Africa with Mediterranean flora. Post-wartime conditions were, however, too unfavourable for the realization of his project. On British initiative the question was discussed at the eighth International Botanical Congress (Paris, 1954) and shortly afterwards a committee was formed in Britain with T. G. Tutin (Leicester) as the chairman and V. H. Heywood (Liverpool) as the secretary. With enthusiasm they went to work. They gained support from all parts of Europe and financial aid from various sources, especially the D.S.I.R. Editors for the different families, regional advisers for the different countries and contributors were appointed. It was soon realized that the inclusion of North Africa would mean a disproportionately large amount of work, and so Europe was taken in its traditional sense. It seems queer, however, that Novaya Zemlya is excluded.

The editors' intention has been to produce a concise and complete Flora in the shortest possible time instead of aiming at the solution of all problems by meticulous monographic studies. The first volume was ready in 1964, and it is planned to complete the *Flora* over the next eight years.

The first volume treats the pteridophytes, the gymnosperms and part of the dicotyledons (Salicales—part of Rosales). Among the larger families may be mentioned Caryophyllaceae, Ranunculaceae, Cruciferae and Saxifragaceae. The largest genera are *Silene* (166 species), *Ranunculus* (131 spp.), *Saxifraga* (123 spp.) and *Dianthus* (121 spp.). It follows from the aim of the work that the treatment cannot be uniform. This seems to be the case especially with the higher taxa. The delimitation of families and genera is sometimes hypermodern but mostly conservative; the generic concept is sometimes wide but mostly rather narrow. *Huperzia*, *Lepidotis* and *Diphasium* are thus segregated from *Lycopodium*, *Consolida* from *Delphinium*, *Hepatica* and *Pulsatilla* from *Anemone*, and *Jovibarba* from *Sempervivum*. The species concept is, on the whole, moderately narrow. Subspecies are included but as a rule not taxa of lower rank. In some cases rather local races (especially some from the British Isles) have been treated as sub-species; in numerous other cases such are discussed shortly without being given a definite taxonomic rank. The descriptions of the taxa of all ranks are clear and concise. There are keys not only to genera, species and sub-species but also to the families, which is a most unusual feature. I have not as yet tried to use the keys, but they look reliable. I have looked up all the Nordic plants and found no serious errors or omissions but a few debatable points and several interesting suggestions which deserve careful consideration. For less-known parts of Europe the volume contains a wealth of new information. In all parts of Europe it will certainly stimulate further research along different lines, as it gives an excellent review of the present state of our knowledge and makes the results of previous studies easily available. Last but not least, it will be the indispensable tool for everybody wishing to name a European plant from outside his own country. The next three volumes are eagerly awaited. All engaged in the project are to be congratulated on this first volume.

J. A. Nannfeldt

Modern Trends in Immunology, I

Edited by Prof. Robert Cruickshank. Pp. vii+263. (London: Butterworth and Co. (Publishers), Ltd., 1963.) 65*s*.

THE rapid developments in immunology to-day undoubtedly call for a 'modern trend' series to supplement the classical text-book treatment of the subject. The horizons of immunology are expanding all the more quickly because of the removal of barriers between different disciplines and the exchange of techniques.

This book is indeed a good start to a series of such modern trends. Although much of the basic knowledge is reviewed, for completeness, the modern views are put into perspective and recent technical developments are well covered. The chapters on immunity have nothing new to add when dealing with natural or acquired bacterial immunity and it is surprising to see so much space allotted to such text-book information. The chapters on acquired viral and protozoal immunity, however, bring the subject up to date. The chapter on immunization "follows a course more or less as empirical as the subject itself" (to quote the author), and it is unfortunate that the reporting of the part played by maternal antibody is not up to date. In this chapter there is much overlap with that on acquired bacterial immunity. Hypersensitivity is well presented and, as the author says, the different disciplines now engaged in this subject may well result in the resolution of many of the problems in the next few years. The chapters on auto-immunity and transplantation immunology are most informative and do much to bring the book into the modern trend. Here again it is noteworthy that the authors comment that "the fusion of transplantation with other immunological disciplines also involves a complementary process".

This book is most useful to the post-graduate student and we hope it is the first of an interesting and informative series. F. T. PERKINS

Electrical Machinery and Control

By Prof. Irving L. Kosow. (Prentice-Hall Series in Engineering Technology.) Pp. xx+707. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1964.) 94*s*.

ONE of the major difficulties that impose unnecessary restrictions for the control engineer designing a complex system is that the specialist designers of the components that go to make up the system are motivated by different criteria. Thus, the electrical machine designer is concerned with iron and copper losses and with stray load losses. While these are the keystones of good design for large power machines when used independently, they are of secondary importance to such factors as controllability, torque to inertia ratio and power gain when used as a component part of a control mechanism.

It is therefore with great expectation that one approaches a new work combining both control and machinery. The connexion is, however, never made; control is represented by a final short chapter on servomechanisms so elementary and trivial as to be useless. Not once in the whole 700 pages are the dynamic characteristics of machines mentioned. The steady-state approach is used throughout and so is of little use to the control engineer. The addition of the term 'Control' to the title of the book is thus misleading.

As a book on machine principles it is also very doubtful if it will arouse much interest. It is written in an elementary manner with no attempt at analysis and a lack of modern fundamental unifying concepts. As an example one could quote the treatment of the force on a conductor carrying current in a magnetic field. The simple law is quoted but there is no discussion on the physical implications. There is no treatment of current embedded in slots in iron and the distribution of forces on the iron, and little evidence for the student that in modern machines the field around a conductor is negligible and that the tooth forces produce the drive.

It is also extremely naïve in its approach. After stating that synchronous induction motors are only made in smaller sizes, it says "Because of the salient pole rotor the motor pulls into synchronism quite easily . . .". This is not true; it is the difficulty of the synchronizing phenomenon (which is a transient dynamic problem and therefore ignored) which prevents large-scale machines of this type from being constructed.

Each chapter concludes with a number of problems and the answers given separately. A set of trigonometry tables is provided as an appendix. JOHN C. WEST

Beryllium Oxide

Edited by R. Smith and J. P. Howe. (Proceedings of the First International Conference on Beryllium Oxide, Sydney, Australia, October 21–25, 1963.) (Reprinted from *Journal of Nuclear Materials*, 14, 1964.) Pp. 498. (Amsterdam: North-Holland Publishing Company, 1964.) 200*s*.

THE first International Conference on Beryllium Oxide, attended by scientists from six countries, was held at Sydney in October 1963. The conference was sponsored and organized by the Australian Atomic Energy Commission to review and discuss recent advances in knowledge of the behaviour of beryllium oxide, particularly from the point of view of its possible use in nuclear reactor systems, as a moderator material.

The present volume, which is reprinted from the *Journal of Nuclear Materials*, is a collection of the fifty-five papers presented at that Conference, together with an account of the discussion which they provided. The papers are divided into seven sections. These deal with: (i) utilization of beryllium oxide in nuclear reactors; (ii) radiation effects in beryllium oxide; (iii) structure and physical properties of beryllium oxide; (iv) sinterability and sintering of beryllium oxide powders; (v) fabrication and mechanical properties of beryllium oxide; (vi) corrosion of beryllium oxide by water vapour; (vii) beryllium oxide-based dispersion fuels.

The book is attractively produced.

M. F. LAPPERT

European Brewery Convention

Report of the Barley Committee: Trials, 1963. Pp. 184. (London: The Institute of Brewing, 1965.) 15*s*.

THIS consists very largely of tabulated results of growth and malting trials of about twelve comparatively new hybrid barley varieties in fifteen Western European countries. The data, mostly arranged country by country but with a broad introductory survey, cover a much wider range than might be anticipated. Thus, they include summaries of trial lay-out, field observations, meteorological conditions as well as the crops themselves and their analytical examination. The objects behind this massive effort are not specifically or solely concerned with those qualities, such as disease resistance, yield, earliness in ripening and production of straw, which might first come to mind. These considerations are all economically important but are in the present context secondary to the main one of malting quality It is clearly unlikely that any one variety will be equally desirable under a wide range of climatic and agricultural conditions. The continuing experience afforded by these trials, nevertheless, must be generally valuable, not least in respect of the economic conditions brought about by the European Common Market. The scientific value of this *Report*, however, encompasses more than the results themselves or the clear way they are presented; it presents what is probably a unique picture of the inevitable complexity involved in selecting improved varieties of even a single crop when many slight or scarcely appreciated variables may be brought into play. A. H. COOK

RADIO OBSERVATIONS OF SPIRAL GALAXIES

By Prof. B. Y. MILLS, F.R.S., and J. R. GLANFIELD

Cornell-Sydney University Astronomy Centre,
University of Sydney

An opportunity to investigate some of the brighter southern galaxies has been afforded by the recent completion of the east–west arm of the cross-type radio telescope at the Molonglo Radio Observatory[1]. While a thoroughgoing investigation requires the resolution of the whole instrument, it is found that the east–west arm alone, operating as a simple total power radiometer, provides quite useful information. It has a beam-width measuring 1·5′ E.–W. by 4° N.–S. at a frequency of 408 Mc/s and is limited in sensitivity by confusion effects at a flux density of about 0·5–1 flux unit (10^{-26} W m^{-2} (c/s)$^{-1}$). Several observational programmes are being carried out while work proceeds on the completion of the north–south arm.

We here report observations of the three brightest southern spiral galaxies, *NGC* 253, *NGC* 4945 and *NGC* 5236. These were first observed by Mills at a frequency of 85 Mc/s with a beam-width of 49′ arc[2]. It appeared that their integrated emission is very similar to spiral galaxies observed in the northern hemisphere by Hanbury Brown and Hazard[3], but the resolution was not adequate to resolve the galaxies and examine their structure. More recent observations by Mathewson and Rome[4] gave surprising results. Using the Parkes 210-ft. reflector at a frequency of 1,410 Mc/s, at which the beam-width is 14′ arc, they found that none of the foregoing galaxies was resolved, although their optical sizes are such that a clearly visible widening of the radio telescope response was expected. They concluded that the model of radio emission comprising a very extensive radio corona, as proposed by Hanbury Brown and Hazard, was not universal, and that in the case of these three galaxies the emission was concentrated close to the nucleus. However, the earlier observations of the similarity in the integrated fluxes was confirmed; it appeared that the total radio emission is closely proportional to the optical emission, irrespective of its concentration to the nucleus.

The Observations

Our new observations are represented by the drift scans shown in Fig. 1. In these, the aerial is pointed at the declination of a galaxy and scanned across it in an east–west direction by the rotation of the Earth. Sidereal time markers are shown at minute and half-minute intervals. Also, under the galaxy records, the east–west extent of the optical image of the main body of the galaxy is shown.

In all cases there exists an extended component of emission roughly co-extensive with the visible galaxy. In addition, both *NGC* 253 and *NGC* 4945 show the presence of a small bright source close to the centre of the extended component. It is unresolved (< 0·5′ arc) in the case of *NGC* 253 and of the order of 0·75′ wide in the case of *NGC* 4945.

Scans were also made at declinations 2° north and south of the galaxy positions. The results were consistent with an origin of the emission entirely within the galaxies, except for the concentration immediately following *NGC* 4945, which may be an unconnected point source at a slightly different declination. For the purposes of analysis it will be assumed to be unrelated to the galaxy, and its contribution to the flux subtracted.

Before discussing our new results in more detail it is necessary to enquire why Mathewson and Rome were unable to detect the extended components. They quoted angular sizes ⩽ 5′ arc, whereas inspection of the records in Fig. 1 shows that the overall east–west extension varies between about 8′ arc for *NGC* 5236 to approximately 18′ arc for *NGC* 253. Probably their results arose as the combination of several factors. In the case of *NGC* 5236 the size is not much larger than their lower limit, so that the failure to observe an extension is probably not significant. Although the extended component is larger in the case of *NGC* 4945, it is quite understandable that the strong central component would dominate the picture. However, the size and integrated emission of the extended component of *NGC* 253 would appear to be easily observable. It seems likely that it was not observed because of a rather fundamental difficulty. The resolution of a radio telescope may be controlled by changing the observing frequency, but, if a radio source is completely resolved,

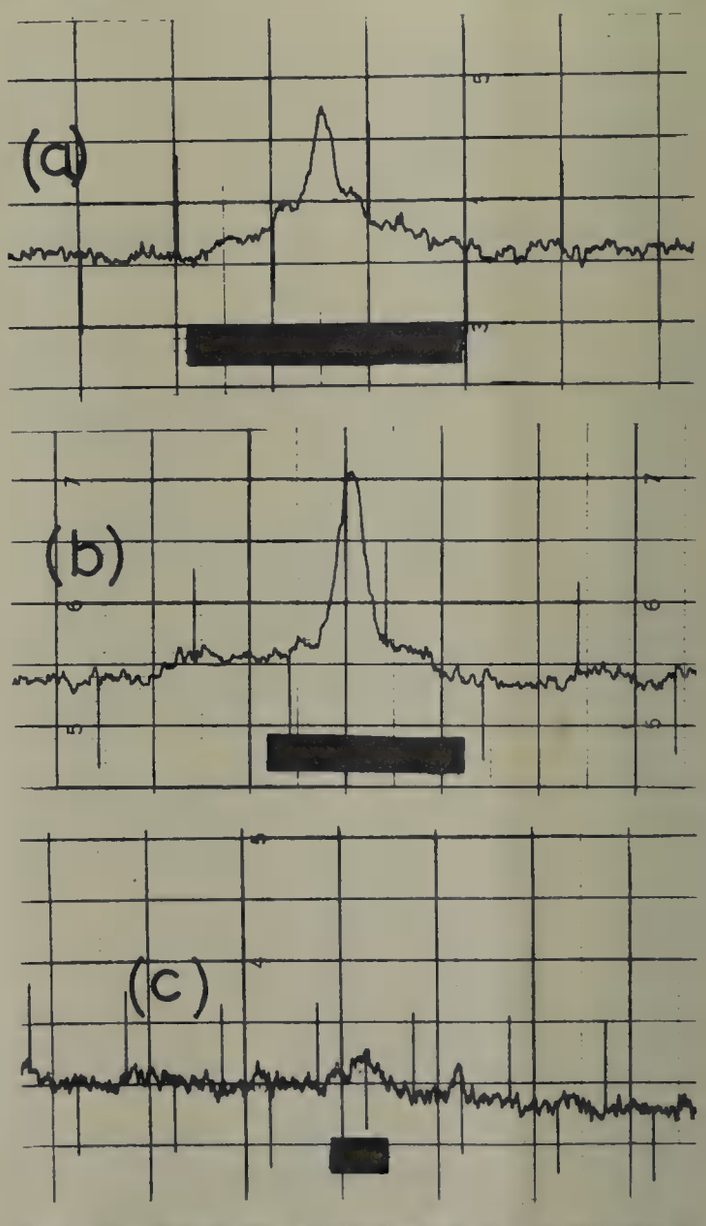


Fig. 1. Transit observations of the three galaxies: (*a*) *NGC* 253; (*b*) *NGC* 4945; (*c*) *NGC* 5236. Minute and half-minute time markers are shown and in each case the east–west extent and position of the main body of the galaxy is indicated by the black line immediately below the chart record. The receiver output time constant is 0·8 sec

the received power is proportional to $\lambda^{2+\alpha}$, whereas the received power of an unresolved source is proportional to λ^{α}, where α lies in the range 0·5–0·7 for the corona of a normal galaxy. Thus, if one increases the resolution by decreasing the wave-length, it becomes increasingly difficult to observe the extended sources which are resolved. This effect is seen in its most acute form in the corona of the Milky Way which, using present techniques, is unobservable at centimetre wave-lengths.

Physical Properties of the Galaxies

Our knowledge of the physical properties of the galaxies comes essentially from the work of de Vaucouleurs. Most information is available about *NGC* 4945, for which values of distance modulus, total absolute magnitude and apparent magnitude are given, together with other photometric data which are not at present relevant[5]. Equivalent data are also presented for *NGC* 253 but not in such detailed form and probably with lower accuracy[6]. Little information is available about *NGC* 5236, and to compare it with the others we have supplemented de Vaucouleurs's data[7] with *The Hubble Atlas of Galaxies*[8]. To obtain a distance scale, we have assumed that the absolute magnitude is the same as *NGC* 253. This leads to a distance modulus close to that of *NGC* 4945, consistent with the suggestion of de Vaucouleurs that they belong to a single physical grouping of galaxies[6].

All the galaxies are late type spirals, the earliest being *NGC* 5236, which Sandage denotes as *Sc/SBb*, and the latest being *NGC* 4945, which de Vaucouleurs denotes as *SB(s)cd*. Their basic properties obtained as here are summarized in Table 1: the magnitudes have been corrected for internal, as well as galactic, absorption.

Calibration of the Equipment

The instrument has only recently been put into operation and calibration programmes are at present being undertaken to place the measurements of flux density and position on a suitable absolute standard. For the present observations the right ascensions have been taken directly from the transit times measured on the uncalibrated instrument. Checks which have been made with lunar occultation sources of accurately known position suggest that errors do not exceed 10″ arc. The declination is defined with an accuracy better than 0·25°.

The flux density scale is not yet in such a satisfactory state, and for these observations our procedure was to check the calibration at intervals by observations of convenient sources in the catalogue of Bolton, Gardner and Mackey[9], which gives fluxes of radio sources at 408 Mc/s based on the scale of Conway, Kellerman and Long[10]. The accumulated uncertainties appear quite large and our relative fluxes are thought to be accurate only to about ± 15 per cent: the uncertainty in the absolute calibration may also be of this order. This degree of accuracy is considered adequate for the observations recorded here.

Analysis of the Observations

NGC 253. The right ascension of the central source is 00h 45m 05s (epoch 1950·0). The position of the galaxy is given as 00h 45·1m (1950) (ref. 3), so that our observations are consistent with a point source placed at the nucleus of the galaxy. The flux density of the source is 3·5 flux units.

Table 1. Properties of the Galaxies

Galaxy	Corrected (*m*pg)	Corrected (*M*pg)	Distance (pc × 10^6)	Radius in plane of galaxy (pc × 10^3)
NGC 253	6·5	−20·5	2·5	8
NGC 4945	7·55	−20·35	3·8	8
NGC 5236	7·1	−20·5	3·4	5

After making allowance for the smoothing effect of the aerial beam, the east–west extent of the extended component is approximately 18′ arc; this corresponds to an angle of 19·5′ arc measured along the major axis at position angle 67°. The integrated flux is 20 flux units.

It is interesting to enquire what spatial distribution of emission would reproduce the observed telescope response. As we have no information about the north–south distribution we have assumed the emission is distributed in a series of uniform spheroidal shells of differing emissivity, centred on the galaxy, and have determined the radial distribution of emissivity along the major axis which best fits our observed galactic profile. A more refined analysis should be possible with the completed instrument.

Using a fan beam aerial which does not resolve the source in a north–south direction, it is easy to show that the radial distribution of emission in a spherically symmetric source is given by:

$$E_\theta \propto \frac{1}{\theta}\frac{dT_\theta}{d\theta}$$

where T_θ is the aerial temperature at the angle θ from the centre of the distribution. With a spheroidal source of unknown ellipticity the same expression may be used to determine approximately the emissivity in the galactic plane at an east–west angle θ from the nucleus. If the ellipticity is large or the major axis nearly east–west, the errors will be small and probably negligible compared with uncertainties introduced by random noise and unresolved sources. It is worth pointing out that the foregoing equation implies that a line scan of an infinitesimally thin spherical shell produces a rectangular response.

The observed profile can be fitted quite well by a three-component model as follows: (1) an unresolved nuclear source with a radius less than 200 pc. and a total emission of $2{\cdot}6 \times 10^{21}$ W(c/s)$^{-1}$; (2) a less-concentrated component of uniform emissivity, radius 2·5 kpc and total emission $5{\cdot}5 \times 10^{21}$ W (c/s)$^{-1}$; (3) an extended component of uniform emissivity, radius 7 kpc and total emission 9×10^{21} W(c/s)$^{-1}$.

Using the same distance scale, the overall radius of the visible galaxy is approximately 8 kpc. A bright central structure can be observed extending out to about 3 kpc, perhaps corresponding to our second radio component. The nucleus is not evident on photographs available to us.

NGC 4945. The right ascension of the central source is 13h 02m 33s (epoch 1950·0), and its flux density is 9 flux units. The position of the galaxy is given as 13h 02·4m (ref. 7). The galaxy is observed edge on and the relatively heavy obscuration hides the nucleus so that the optical position must be judged from the rather irregular brightness distribution. The agreement is sufficiently good to be sure that we are observing the galaxy.

We have analysed the extended component in the same way as before, but this time find that a single ellipsoid of uniform emissivity is adequate to fit the observation. The total flux density is roughly 10 flux units, with some additional uncertainty because of the small source close to the galaxy.

Thus, after allowing for a position angle of 42°, the observations may be represented by the following two-component model: (1) a central, presumably nuclear, source with a radius of the order of 400 pc and total emission 15×10^{21} W(c/s)$^{-1}$; (2) an extended component of uniform emissivity with a radius of 8 kpc and a total emission of 17×10^{21} W(c/s)$^{-1}$.

The radius of the visible galaxy is also about 8 kpc so that the second component here corresponds to the third component in *NGC* 253, both having the same extent as the visible galaxy. An intermediate component may be present in *NGC* 4945, but it is not separately distinguishable.

NGC 5236. This galaxy is in a rather confused region of several radio sources. Nevertheless, a small extended

source at the galaxy position is quite clear. There is no obvious nuclear component so that it is difficult to quote an accurate position. The centroid of the whole source is approximately 13h 34m 11s, which agrees well with the galaxy position of 13h 34·3m (ref. 7). The integrated flux density of the whole galaxy is 5 flux units and its overall extent, after allowing for the aerial resolution, approximately 8′ arc. In this example the galaxy is seen face on so that there is no need to allow for the position angle of the major axis.

Because of the poor signal-to-noise ratio and the presence of confusing sources, an attempt to separate different components is not justified. Although there does appear to be evidence of fine structure, a simple model comprising an ellipsoid of uniform emissivity fits the observations reasonably well. The total emission is 6×10^{21} W(c/s)$^{-1}$ and the radius of the distribution approximately 4 kpc. As this is close to the radius of the visible galaxy, the component appears to correspond with the extended components in the other galaxies.

Discussion

We have found that all three galaxies examined have extended components of radio emission co-extensive with the visible galaxy and of the same order of total emission. The ratio of optical emission from the whole galaxy to the radio emission of this component is also roughly the same in all cases. Additional more concentrated components exist in two of the galaxies, but not in any fixed ratio.

Apparently similar components have also been recognized in the Milky Way, where we have a nuclear source, a disk component with the greater part of the emission confined within a radius of about 6 kpc and, finally, a corona of approximately uniform emissivity extending to about 12 kpc in the galactic plane[11]. The average overall extent of the Milky Way itself, including the Perseus arm, must be about 13 kpc.

It appears to be very reasonable to identify the Milky Way corona with the extended components found in the other galaxies: the disk and nuclear components at the distance of these galaxies would be too weak to be detectable. Both *NGC* 4945 and *NGC* 253 have, by contrast, very strong nuclear components. In order to avoid postulating an additional galactic component, it also seems reasonable to identify, tentatively, the intermediate extended component of *NGC* 253 with the disk component of the Milky Way. Although this component is relatively much stronger in *NGC* 253, it appears to be related to the visible structure of the galaxy as in the Milky Way.

In Table 2, we accept these suggested identifications and compare the galaxies directly. Values for the disk and corona of the Milky Way have been taken from the 85 Mc/s results[11], assuming an emission proportional to $\nu^{-0.6}$ and a distance to the nucleus of 10 kpc. The emission

Table 2. Radio Properties at 408 Mc/s

	NGC 253	*NGC* 4945	*NGC* 5236	Milky Way
Nucleus: emission W(c/s)$^{-1} \times 10^{21}$	2·6	15	Undetected	0·04
Radius pc	< 200	~ 400	–	200
Disk: emission W(c/s)$^{-1} \times 10^{21}$	5·5	Undetected	Undetected	0·5
Radius pc $\times 10^3$	2·5	–	–	6–10
Corona: emission W(c/s)$^{-1} \times 10^{21}$	9	17	6	7
Radius pc $\times 10^3$	7	8	4	12
Visible galaxy Radius pc $\times 10^3$	8	8	5	13
Radio index M_{408}–Mpg				
(*a*) Corona	+2·4	+1·5	+2·7	+2·6*
(*b*) Whole galaxy	+1·7	+0·8	+2·7	+2·5*

* Assuming the Milky Way has the same absolute magnitude as *NGC* 253, that is, Mpg = −20·5.

of the nucleus itself has been obtained directly at 408 Mc/s using the present instrument.

Although these results make a satisfactorily coherent picture, it appears that the only other spiral galaxy to be studied in detail, the Andromeda Nebula, has much more extensive radio components, although the total emission is similar. Observations at 408 Mc/s using the Jodrell Bank 250-ft. reflector[12] may be compared directly with ours if we assume a distance of 8×10^5 pc to the galaxy[8]. After allowing for the beam-width, the results appear to indicate a coronal component of total emission 10×10^{21} W(c/s)$^{-1}$ extending to a radius of about 40 kpc along the major axis, and a disk component of emission $0{\cdot}6 \times 10^{21}$ W(c/s)$^{-1}$: the extent of this component is uncertain as it was not completely resolved, but it is probably of the order of 12 kpc along the major axis. No nuclear component was detected. The main body of the galaxy, however, has a radius of only about 14 kpc.

Thus, if this interpretation of the observations is correct, it appears that the distribution of coronal radio emission in the Andromeda Nebula is not similar to the distribution in the other spiral galaxies. Clearly, it will be necessary to observe many more galaxies with high resolution to establish the pattern more reliably.

This work was supported by a grant from the U.S. National Science Foundation.

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WILSON'S THEORY OF ICE AGES

By JOHN T. HOLLIN
Department of Geology, Princeton University, New Jersey

A PAPER by A. T. Wilson[1] suggests that the Antarctic ice sheet is unstable: that it builds up until it begins to melt at the base, then 'surges' rapidly outwards to form a large floating ice shelf in the Southern Ocean. Wilson suggests that, in the Tertiary, shelves formed in this way periodically displaced enough sea-water to account for the cyclothems of that time; and that in the Quaternary, helped by some longer-term reduction of temperature, they periodically cooled the oceans enough to cause ice ages in the northern hemisphere. A paper by me[2] points out that, if these shelves did cause ice ages, traces should exist of (otherwise anomalous) rapid but temporary rises of sea-level, of 30–100 ft., just before each ice age. This present article discusses the available evidence for and against such rises. Up to now, most of the evidence seems favourable to Wilson's theory.

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Contains a variety of formulae for computing elementary mathematical functions, representing those functions in the form of power series; series of polynomials (orthogonal and otherwise), continued fractions, limits of iterative processes etc. It also contains approximate formulae for computing the elementary functions to a specified accuracy, and a wide selection of material concerning the elementary functions, organized systematically according to the form of the function. Particular attention is given to formulae which are used in computational practice for working on electronic computers.

Contents: Rational and power functions; Exponential and logarithmic functions; Trigonometric, hyperbolic, inverse trigonometric and inverse hyperbolic functions; Alogarithms used for computing elementary functions on some Soviet computers; Special polynomials and other functions; Numerical tables.

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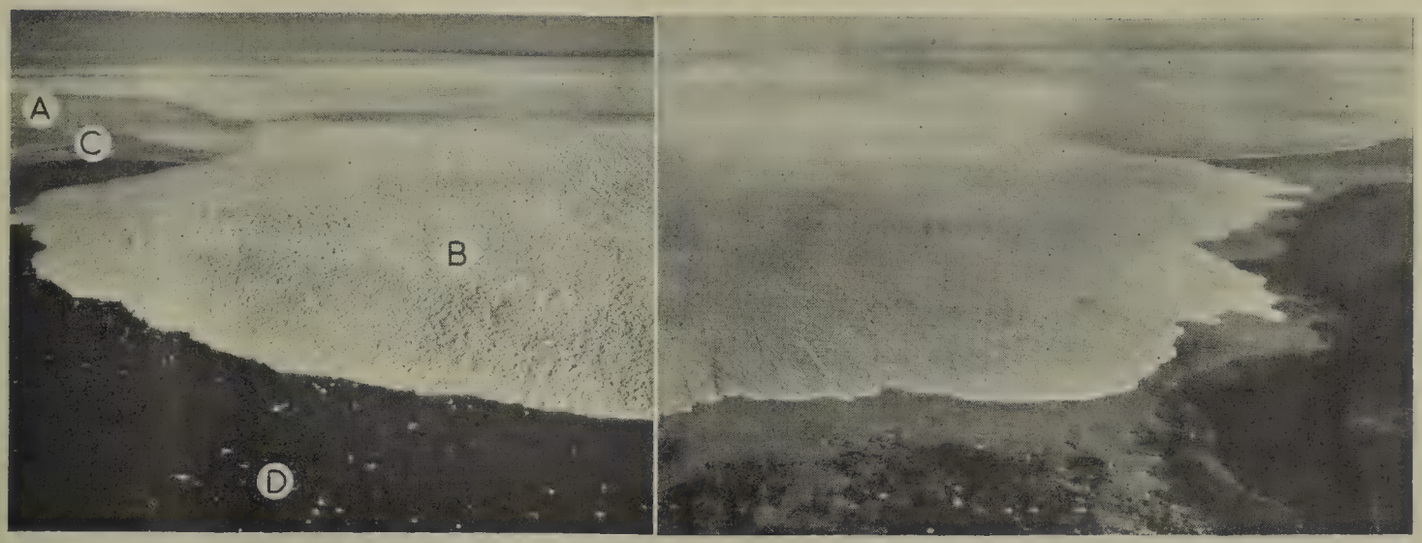

Fig. 1. Bråsvellbreen, looking northwards. Only the western and eastern edges (30 km apart) are shown. *A* (and to the right), 'normal' ice cap; *B*, Bråsvellbreen; *C*, moraines and rocks; *D*, Barents Sea. (*Photographs by Norsk Polarinstitutt*, 1938)

probably that of the Bråsvellbreen (a sector of a roughly circular ice cap in Spitsbergen), which advanced up to 21 km on a front of 30 km, some time between 1935 and 1938 (ref. 4) (Fig. 1). The fastest surge measured so far is probably that of the Kutiah Glacier[1], at 110 m/day, although hearsay surges include one so fast that it overtook two villagers fleeing from it[5]. The cause of surges is unknown. In Robin's theory, which Wilson quotes, surges occur when the continuing build-up of the glacier allows geothermal and frictional heat to bring the base to the melting-point. Once melting begins, large glaciers may find it difficult to expel the large amounts of water they generate and, in Weertman's theory[6], it is the resulting basal water layer which accounts for the high speed of the surges. Now, without further work on these and other theories, it is really impossible to say whether or not surges should occur in large ice caps and ice sheets, and I have personally been somewhat sceptical about such surges[7,8]. However, so as to test Wilson's theory, in order to know what to look for in the geological record of sea-level changes, it is worth while trying to estimate how large and how fast his surges would have to be. My estimate that they would have to involve sea-level rises of 30–100 ft. was based mainly on the assumption that surges smaller than this would have too short a period to fit the time-scale of ice ages, and on the fact that deglaciations larger than this are almost impossible to imagine glaciologically, at least without moving Antarctica northwards. To illustrate this, we can refer to some of the known facts from Antarctica[7]. The current average thickness of the ice sheet there is roughly 2,300 m. Suppose that this builds up to 2,400 m. Suppose that a surge emerges from either Wilkes Land or the Filchner area, captures the drainage[9] from half the ice sheet, and lowers that half by 50 per cent. Such a surge, spread out to the typical ice shelf thickness of 200 m, would reach 55° S and, as Wilson points out, would appreciably increase the Earth's albedo. The central thickness of the ice sheet is roughly 4,000 m. The central accumulation rate is roughly 3 g/cm² yr, so that a central lowering by 50 per cent would take approximately 70,000 yr to build up again. Very roughly, this would be the period of the surge. The surge just described would be in the middle of my estimated range: ignoring isostatic effects, it would raise sea-level by 65 ft. On the speed of the surges, if they are in fact a cause of ice ages, then they must certainly be rapid to overwhelm the great capacity of the Southern Ocean for breaking up and melting Antarctic ice[10]. It is interesting, therefore, to realize that if the ice in the centre of the ice sheet began to surge with the velocity of 110 m/day quoted above, it would in fact reach the edge of the ice sheet in only 40 yr.

These estimates of the size and speed of Wilson's surges are incorporated in Fig. 2. Fig. 2 (1) shows the conventional view of sea-level change during ice ages such as the Weichsel (Würm, Wisconsin), with the Antarctic ice sheet remaining roughly constant in volume. Fig. 2 (2) shows the sea-level change required by Wilson's theory in Tertiary time: rapid rises accompanying the surges from Antarctica, and slow falls accompanying the build-ups there. Fig. 2 (3) shows the change required for Quaternary time: a rise accompanying each surge, a fall as both the Antarctic and northern ice sheets build up, the end of the ice age and a levelling out as the northern ice sheets disappear, and a subsequent small fall accompanying the final build-up in Antarctica. Although these estimates are very speculative glaciologically, they form a very specific test geologically. The chances seem small that rapid rises of sea-level of 30–100 ft., repeated in several interglacials at precisely the break of climate, could be caused by anything other than Antarctic surges.

As might be expected, the most detailed evidence for and against the sea-level changes of Fig. 2 (3) comes from the uppermost, most-studied part of the Quaternary, especially from north-west Europe. In this area, sea-level movements are recorded by changes in the land- or sea-shell content of deposits, and the date of the movements is recorded by the pollen content. Each of the last few interglacials in north-west Europe can be divided now into several pollen zones, and this permits very accurate dating. Within north-west Europe, the best areas for an investigation of this type appear to be southern England and northern France, where the horizontality of old, high-level marine features suggests that these areas are essentially stable. Not so many deposits have been examined there as in Holland and Germany, but in those areas there has been considerable warping, and it is difficult to reconstruct the old elevations involved. Fig. 3, therefore, is an attempted reconstruction of sea-levels during the last two interglacials in southern England. (For the Hoxnian interglacial, it has been necessary to

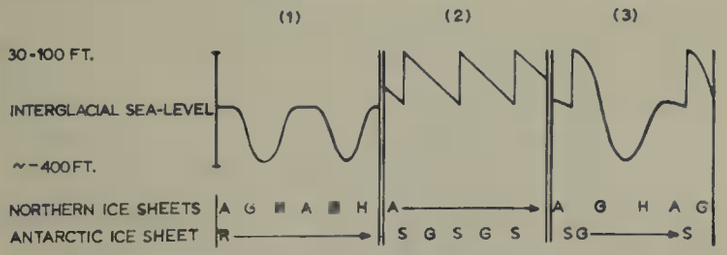

Fig. 2. Three patterns of sea-level change. The time and elevation scales are compressed in the ice ages. *A*, absent; *G*, growing; *H*, shrinking; *R*, roughly constant in volume; *S*, surging

use some data from eastern England, and in this particular case the possibility of warping is discussed below.) Sea-level positions suggested by field studies are plotted, and an attempt is made to connect them with a curve of the Fig. 2 (3) type. How well this curve fits the data is discussed in the following four paragraphs. For those interested, the pollen zones and the sites of Fig. 3 are discussed more fully in papers by West[11] (note that, in ref. 11, the legends of the key Figs. 12 and 13 should be interchanged).

In Fig. 2 (3), the sea-level just before a surge changes only slowly, whereas after a surge it changes rather quickly, because not only the Antarctic but also the northern ice sheets are now building up. Therefore, marine benches are more likely to be cut at a primary, pre-surge level than at a secondary, post-surge one. Accordingly, to agree with those authors who argue that a marine bench between 5 and 30 ft. O.D. in south-west England and north-west France must belong to the Hoxnian interglacial[11,12], I have attributed elevations in the middle of this range to the primary Hoxnian level in Fig. 3. What is striking is that the pollen data from the Hoxnian show that late in the interglacial, at the end of the main warm zone *2*, apparently just as the climate broke, a remarkable transgression occurred in eastern England. The transgression has been recorded at 9 ft. at Clacton[13], at 21 ft. in the Nar Valley (where marine deposits continue to at least 65 ft.)[14], and at 80 ft. at Kirmington[15]. The record of freshwater sedimentation at Southelmham[16], at roughly 80 ft., ends at the end of zone *2*, and would fit a transgression at that elevation at that time. It seems reasonable to assume that the upper limit of the transgression was the 100-ft. 'Tyrrhenian' beach and 'Boyn Hill' terrace level traditionally assigned to the Hoxnian in southern England and elsewhere. The fact that the transgression occurred at all these different elevations at the same time suggests that it was unusually rapid. This rapidity makes it very unlikely that it was due to downwarping. For example, let us, to avoid the effects of tilting, focus our attention on the one small area of the Nar Valley[14]. The 21-ft. difference in elevation between the uppermost zone *3* marine sediments at Summer End and the uppermost freshwater sediments at Horse Fen suggests that at least that amount of transgression must almost certainly have occurred in that relatively short zone. It is difficult to suggest what could have caused a downwarping at this rate, which has obviously not been maintained since. That the transgression at Clacton was the result of a eustatic rise of sea-level rather than of downwarping has already been argued, by Oakley and Leakey[17]. They claim that, for the Clacton area, the rise to the Boyn Hill level is represented by the gravels at 85 ft. at Upper Dovercourt.

In the last, Ipswichian (Eemian, Riss-Würm, Sangamon) interglacial, early in the main warm zone *f*, the sea is known to have risen above −5 ft., at Selsey[18]. Presumably this was the end of the post-Riss rise, because appreciably later in zone *f* the sea was still at −2 ft., at Stone[18]. By the end of zone *f*, river flood deposits were being laid down at −3 ft. at Trafalgar Square[19], which suggests that the sea had by now fallen a little below that level. Now, exactly when the Ipswichian climate began to deteriorate is difficult to say, but it was almost

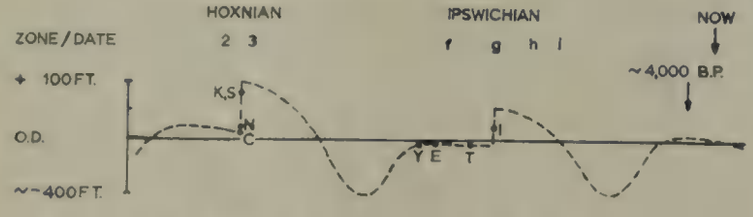

Fig. 3. Sea-level changes suggested for England. The time and elevation scales are compressed in the ice ages. The dots refer to pollen studies: *C*, Clacton; *N*, Nar Valley; *K*, Kirmington; *S*, Southelmham; *Y*, Selsey; *E*, Stone; *T*, Trafalgar Square; *I*, Ilford

certainly in zone *g*[20]. What is striking is that in that zone a transgression occurred in north-west Europe. The transgression is very well documented in north-west Germany[21,22] and, elsewhere, it seems to have formed the Eemian 'Portlandia Sea' of the Baltic[23] and the 'marine intercalation' of Holland[24]. The German pollen data in particular favour a transgression in the first half of zone *g*. At about this time in England, a remarkable aggradation occurred in the Thames estuary where, at 23 ft. at Ilford[25], pollen-bearing sediments were covered by the well-known Thames 'brickearth' or silt. Because of the low percentage of *Carpinus* in the uppermost sediments at Ilford, West *et al.* attribute them to the end of zone *f*. However, because low percentages of *Carpinus* can sometimes occur in zone *g*[20], and because, otherwise, the percentages of *Pinus*, *Quercus* and *Corylus* in the uppermost sediments are quite typical of that zone, it seems quite likely that all these north-west European pollen dates reflect the same event. At Ilford, the aggradation of the brickearth continued to 42 ft., and at other places in the estuary to 50 ft. Since the gradient of the Thames and its terraces in this area is less than 3 in./mile (ref. 26), it seems quite possible that the aggradation of the brickearth was in fact a response to a rise of the sea to the 50- or 60-ft. 'Taplow', 'Main Monastirian' level traditionally assigned to this interglacial. That the Main Monastirian level may indeed have been merely a secondary one (from which the sea immediately began to drop to its Würm level) is suggested by the fact that it is only rarely a bench level, but is chiefly a beach and river terrace level[27]. Similarly, in North America, although the coastal Cape May formation, assigned now to the last interglacial[28], was attributed by Salisbury and Knapp to a sea reaching 50 ft. or so, they remarked that only faint terraces rather than coastal cliffs were to be found at that level[29]. Of course, as well as the 55-ft. Main Monastirian, a 25-ft. 'Late Monastirian' level[26] is often assigned to the last interglacial. This 'double' level is a problem for any theory of ice ages, but, according to Fig. 3, the Late Monastirian level might represent the brief reoccupation of the primary Hoxnian level by the regressing Hoxnian and Ipswichian seas. The Ipswichian regression across O.D. in zone *i* was recorded in northern France[18].

The Ipswichian interglacial provides the only evidence I have been able to find that is plainly incompatible with the reconstruction in Fig. 3. In eastern England, the Histon Road, Cambridge, deposit[30] lies at 15–42 ft. O.D., but shows terrestrial as opposed to marine sedimentation between zones *g* and *i*. Now, because this is the only deposit of its kind so far, it is possible that it is deceptive in some way. One possibility is that the unconformity 500 cm below the top of the deposit, that is, at 25 ft. O.D., does represent a marine interval. The sediments below 500 cm contain high percentages of *Quercus* and *Corylus*, and could quite easily belong to the first part of zone *g*. Then, as Sparks and West point out, a true zone *h* is missing in the deposit, and it is possible that sedimentation above 500 cm did not begin again until zone *i*. If so, the unconformity would fit Fig. 3 exactly. Other East Anglian evidence, such as the 55-ft. Ipswichian terrace at Cambridge[31], certainly fits Fig. 3. However, although the data from Histon Road are insufficient by themselves to disprove the reconstruction of Fig. 3, they do emphasize one of its chief weaknesses: the shortage of really high quality evidence for an Ipswichian sea-level above O.D. As we have seen, the north European data, the Thames evidence and the Monastirian tradition do favour such a level. However, evidence that would really favour Wilson's theory would be aggradation deposits similar to the Ilford ones in other estuaries, or, better, zone *h* marine deposits in the 20–40 ft. range. Because a late Ipswichian sea-level rise would have been short-lived, and the marine extension shallow, and because any deposits in it would afterwards have been

leached, frost-heaved and eroded for the whole of the Weichsel, such deposits, if they exist, may be hard to find. On the other hand, if they cannot be found, and if more deposits such as the Histon Road one are discovered, then these latter would be strong evidence against Wilson's theory, at least as it applies to the latest ice age.

In the current interglacial, since the northern ice sheets disappeared, Wilson's theory requires that sea-level should be falling slightly, as the Antarctic build-up continues. It is possible, of course, that a fall is being offset by continuing glacial retreat in the Canadian archipelago or Greenland, or even by minor surges from Greenland or Antarctica. However, there are many papers which do document a falling sea-level over the past few thousand years[32,33]. On the other hand, a probably larger set of papers describes a rising sea-level[34]. At present, the difference between these results cannot be explained, although it may be due, for example, to local land movements[33]. In this situation, an indication of the world-wide average movement of sea-level might be given by the change, if any, in the Earth's rate of rotation. Analysing the change suggested by the data from ancient eclipses, Munk and MacDonald[35] conclude that there may have been a fall of sea-level of 2 m over the past 2,000 years, and this of course would favour Wilson's theory. A fall of 2 m in 2,000 years would be equivalent to a continuing 25 per cent surplus of accumulation over ablation in Antarctica (assuming that the area of the ice sheet is 1/30 that of the oceans, and the average accumulation on it 14 g/cm^2 yr)[7]. This is larger than the 5 per cent surplus which, ideally, is all that would be required to replace the late Ipswichian surge of Fig. 3 over the roughly 100,000 yr since it occurred. However, the figure of 5 per cent would be bigger if there have been minor surges since the late Ipswichian one. (Such surges could have been one cause of Weichsel 'stadial' advances.) Glaciologically, even a surplus of 25 per cent cannot really be distinguished yet in Antarctica, because our knowledge of ablation rates there is so very limited[8]. The known activity of the ice sheet at the edges of Antarctica suggests that if any build-up is occurring it must be concentrated in the centre of the continent. Evidence for or against this may come in a few years from Franco–Soviet and other strain measurements in that area: evidence that the ice is stretching at less than the rate required to dispose of the snow accumulation would support Wilson's theory.

So far, I have said little about the speed of the sea-level rises. Obviously, our first problem is to find out whether they really occur or not. There are two reasons, however, for saying a little more about their speed. First, Wilson's theory implies that the current interglacial will end with a new surge, perhaps suddenly, some time between now and say A.D. 50,000. Since, from Fig. 3, the sea might rise then by 75 ft. or so, the speed of the rise might have important human implications. Second, chiefly for the purpose of drawing Fig. 2, I mentioned a possible surge time of 40 yr. However, a sea-level rise much faster than this might have left some very unconventional deposits, and unless this is appreciated, these deposits may remain unrecognized. To illustrate this point, let us look, for example, at the brickearth, laid down during the late Ipswichian aggradation in the Thames estuary. The brickearth consists chiefly of silts, which are locally 30 ft. thick but which, unlike the alluvium of the current interglacial, are quite devoid of layers of vegetation, developed during intervals of subaerial exposure. These silts are famous for their numerous, curiously intact remains of large mammals. Many geologists have argued that the silts must have accumulated, and the mammals drowned, during floods much greater than those known to-day[36]. The brickearth is extremely patchy and, indeed, the alluviation of the whole Thames estuary, from below −3 ft. to above 50 ft., would represent an enormous amount of deposition for the end of an interglacial, when if anything the Thames should have been incising rather than aggrading. Also, despite their situation, the shells in the brickearth are overwhelmingly freshwater ones, and only rarely estuarine ones. So long as Wilson's theory is being discussed, the possibility ought to be borne in mind that in fact the brickearth never was a normal alluvium, but was merely the soil and fauna of zone *g*, re-worked by a very rapid rise of sea-level, and swept into the embayments of the marine extension. Only after this diluvium had settled would conventional estuarine and marine deposits have begun to accumulate above it and, as we saw two paragraphs ago, such deposits may be hard to find. (The same possibility ought to be borne in mind for the brickearth at Stutton[11,37], where the evidence is otherwise incompatible, though not decisively so, with the curve of Fig. 3. Note that the data from nearby Bobbitshole[38] fit Fig. 3.)

Even if these late interglacial transgressions do prove to have been very rapid, determining their precise velocity may be difficult, except in one eventuality. As opposed to the Kutiah velocity in the second paragraph, the hearsay velocity (presumably several km/h) yields a surge time of only 1 week. Now a surge in only 1 week or so would involve a very rapid redistribution of ocean water, and the question arises whether or not the sea-level rises might have occurred in the form of waves or 'tsunamis', such as sometimes run several miles inland when they break on the coasts of the present-day Pacific Ocean[39]. This is a possibility that can be tested, as follows. Let us look this time at the Hoxnian interglacial, for which a secondary, post-surge coastline of 100 ft. was suggested. If waves occurred, sediment-pollen studies some way inland from that contour should detect some disturbance at the time of the surge. The waves would be unlikely to have left any marine traces, such as shells, but they might have left a layer of debris, such as gravel or tree trunks. So far, I have been able to find only one investigation from such a situation, from Kilbeg[40] in southern Ireland. The profile there does show an unconformity covering the break of the Hoxnian climate, but the gap covers too long a time to be good evidence for waves. However, if further investigations in such situations can be found or made, it should be possible to complete this test.

If all large glaciers are indeed susceptible to surges, either because of the instability discussed above or, though this seems less likely, because of some external cause, then this raises many interesting questions. One of the most interesting must be whether or not surges of the Gondwanaland ice sheet may account for the Carboniferous cyclothems, especially for the rapid submergence of the coal beds. However, although these questions do deserve our attention, it looks as though the most rigorous progress in the matter of surges will be made by concentrating on Wilson's theory of Quaternary ice ages, particularly on events late in the last interglacial. As was mentioned earlier, the sea-level test for Wilson's theory is a very specific one, and it is certainly intriguing to find these traces of late interglacial transgressions with exactly the required timing, amplitude and speed. One explanation which might be advanced for them would be that they were merely continuations of the main post-glacial transgressions, and due either to a normal (as opposed to surge) deglaciation of Antarctica by the continuing warmth of the interglacial[7], or else due to local downwarping. Another explanation would be that they were merely recoveries from a regression due to a cold spell in the middle of the interglacial. Up to now, Fig. 3 and the foregoing discussion are strongly against these explanations, and are favourable to the Wilsonian explanation. There is clearly a need for more data, but there seems little doubt that further work along these lines will complete the test of Wilson's theory.

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"THERE IS ALL AFRICA"

By Dr. A. S. THOMAS

EVER since the first explorers penetrated the centre of Africa, Europeans have desired to bring peace and prosperity to the continent; the most striking proof of that desire has been the devotion and self-sacrifice of the missionaries. But there was often a bland assumption that European methods of clothing, farming and political organization could be applied without modification near the equator. In courses of training for work in the tropics there was much emphasis on primitive agriculture —unchanging and resistant to change.

But, as a farmer's son who had done much hard work, some of which seemed unnecessary, I found myself thinking how sensible the Ghana farmers were to grow their cocoa crops with a minimum of work. Their mixtures of food crops looked untidy after European monoculture, but many of these crops had been introduced to Africa, some in the far past, some recently—bananas and cocoyams from Asia, cassava, other cocoyams, maize and sweet potatoes from America—and their culture must have involved great changes.

The Krobo farmers explained that their farms were in strips across the valleys because that was the only fair way of dividing the land—there was one kind of soil on top of the hills, one on the sides and one in the valleys. That important point had not been mentioned in any of the lectures on soil science to which I had listened, though the same sense of values had led to division of much of southern England into long strips of parishes. My appointment in Ghana lasted only for one tour; money was short in 1930 and there was considered to be no need for research on cocoa; six years later a Ghana farmer took specimens of swollen shoot to the Division of Agriculture and asked why his cocoa was dying.

On arrival in East Africa, I found that the soil chemists were studying soils in series or catenas. Most of Africa is a vast peneplain of old land surfaces dissected by water-courses; there are few level areas except the plains of dark clay which are under water for part of the year, dry and deeply cracked for another part; even these plains show slight differences in levels; parts which are submerged for much of the year must be considered in conjunction with the higher ground on which wild and domestic animals graze during the rains.

People are taught about Africa from small-scale maps which cannot show the undulating surface or the variety of vegetation associated with it; then some people go to Africa expecting to see vast stretches of virgin forest, their false ideas having been reinforced by travel films—"And so we set off through the African jungle", taken in a place where there is no jungle, only the coffee bushes shown on the screen. Even when African forests are tall, they may be quite young; a patch in the Botanic Gardens, Entebbe, Uganda, is so fine that it has been used as the setting for a "Tarzan" film; but a photograph shows that fifty years ago it was a grove of raphia palms without any trees.

Many theories have been postulated about tropical forests on the assumption that the areas examined were climax communities when, in reality, they were unstable young phases of forest growth. Even the woodland areas have been represented as climaxes, with maximum energy flow; the exposed strata on any river bank of such an area will show that there has been no stability; the layers of water-borne and wind-borne deposits prove there has been no stable state, only a sequence of changes.

Philosophical phrases such as "Man in harmony with his environment" would not mean much to an African peasant whose blood is invaded with malaria parasites, whose farm is invaded by elephants, monkeys and wild pigs. When you have recurrent dysentery and malaria you come to regard your body, not as an individual, but as an ecosystem; one man, who had a tapeworm as well, used to say: "We have enjoyed our meal".

Conservationists talk and write of man as a destroyer, not mentioning the way in which man builds up the soil of his settlements. There are many proofs of this building-up: the depth of deposits in old European cities, the huge mounds which cover ancient cultures in the desert areas of Africa and Asia, and the deep layers of broken pottery on some hill tops in the wetter parts of Africa.

Furthermore, the soils of settlements are richer than those of the country around them. Saltpetre used to be extracted in India from the soils of places where there were dense populations of men and domestic stock, and a residue of ashes from wood fires—conditions which can be matched in Africa. Africans select former village and

cattle kraal sites for the cultivation of crops like bananas and tobacco which demand high fertility. The banana is a most useful indicator plant in the wetter parts of Africa, for it will flourish and persist only on rich soils like those of the western slopes of Mount Elgon which have an agricultural population of several hundreds, even more than a thousand, per square mile.

If a house of wood and grass is deserted, it soon rots away under an equatorial climate; no trace will remain above ground, and the only clue will be in the soil—the local accumulation of bases, especially of lime, which is easily detected by tests for soil acidity. Some crops may benefit from the presence of bases; others, such as the tea plant, will die out in patterns to show the plans of former villages, proved by abundance of potsherds.

Certain trees often indicate former settlements; in the drier parts of Africa, the baobab, *Adansonia digitata*, is largely confined to past and present settlements; in the woodland areas, groups of very large shea butter trees, *Butyrospermum parkii*, are to be seen in the villages, or where the villages have been.

Chlorophora excelsa, a magnificent tree of the forest zone, is associated with the spirits of ancestors both in East and West Africa; the belief seems founded on the fact that the tree needs rich soil and protection from fire, conditions usually only found on farms. Soil tests near many *Chlorophora* trees on farms, in forests and woodlands, always showed the soil to be more alkaline near the tree than at a distance from it, where the soil often was too poor for the tree to grow. Yet this tree has been used to denote plant associations without mention of its restricted growth or of the unsuitability of the soil for its growth. It is restricted to settlements, just as in many grasslands the shrubs are restricted to the termite mounds, the soil of which is less acid than the rest of the land.

Diversity of soil is a usual feature in Africa; soils on the hills are always different from soils in the valleys, and the incidence of human settlements and termite mounds is likely to give diversity to the soils in any single zone; hills not very far apart may show distinct differences in their soils and in the way in which they are used[1]. "In Ghana, soil series rarely cover a sufficient area in individual expanses to make it practical to map them on any but very large-scale maps"[2]. Yet there are still being produced maps which show generalized arbitrary classifications; it is most interesting to consider places which one knows well, to compare the different classifications by people who may or may not have visited the area and to think how untruly these classifications represent the actual vegetation.

There are many difficulties in African ecology. More than twenty years ago I discussed the difficulty of learning the names of the plants with another man and found that he, like me, after two years in Africa had felt that he would never be able to learn the names; but that, again like me, after three years suddenly found that he knew enough names to make ecological investigations without taking a large number of specimens for determination in the herbarium. We were both quite intelligent, both very hard-working; and I refuse to believe that much good work could be done by those who write descriptions after a few weeks in Africa, looking at plants they had not seen before.

Furthermore, as Buxton showed, the aspect of any small piece of African vegetation alters completely with the seasons[3]; the eye selects those species which are in flower at the season when they are viewed. So I made it a rule never to publish an account of any patch of vegetation unless I had visited it at different seasons and had described it without reference to previous notes. Yet many of the papers describe large areas after very short visits; if work is done carefully and factually, it must be confined to a small plot; and there is no justification for extending such a description over a region. Yet once a description is in print, it is regarded as factual and is reproduced in the text-books.

The ease and rapidity of modern travel only add to the unreliability of some accounts founded on observations from a motor-car. A road may seem to pass through dense forest when in reality there is only a screen of trees hiding the farms and villages; but in open country a road may pass through a string of farms although the land away from the road is not cultivated. An expert whom I was escorting through Uganda once stopped the car and said: "You have told me that *Cynodon dactylon* is not important in the natural grassland of this region; we have been through a belt of it, fifty miles wide". I tactlessly suggested that the belt was fifty miles long and ten feet wide, as he could see if he got out of the car; he did not, and he did not speak to me for the rest of the day as he was so angry that neither I nor the grasses conformed to his ideas.

In the old days, when aeroplanes flew low and slow, ecology could be done from them, but that is seldom possible from modern jet airliners. But air photographs are a most valuable means of survey; they show intricate patterns of fields, fallows, grasslands, woodlands and forests which give a truthful picture of the countryside, quite different from the generalized subjective accounts so often printed. The camera is objective.

One thing which horrifies visitors to Africa is the intensity of the fires which sweep through woodlands and grasslands. But Africa has been burnt over for many hundred thousand years; some fires are started by lightning and, long before there was any farming, fires were used in hunting and collecting honey. On my first visit to Mount Kadam, my Karamojong porters were astonished at my anger when they burnt a patch of forest to get a pound or so of honey, and they thought me fussy when I made them extinguish every spark. They looked on fire as part of the climate, and so do many African plants; a large living collection of Uganda grasses dwindled under cultivation, in spite of careful weeding; but when, in desperation, I set fire to them, they responded with normal vigorous growth.

There has been much outcry about the destruction of organic matter, but agricultural scientists are now agreed that burning is the only feasible way for the African farmer to get rid of rubbish before planting his crops. The burning of grassland has been condemned, but many people are coming to realize that it is the only practicable way to clear the mass of dry inedible stems and to produce flushes of young leaves for livestock during the long dry seasons. The wild animals, especially the smaller antelopes, also benefit from this burning; they dare not venture into tall unburnt grass, for fear of predators, and often may be seen grazing close to the cattle; the films usually show them on short young grass but do not show the cattle. Furthermore, in controlled experiments it has been found that the soils of grasslands burnt every year may contain more organic matter than do the soils of unburnt forests on similar ground.

Where there are cattle in Africa, there is usually erosion. It is not the treading of cattle which has attracted attention, but the grazing; yet in reality the treading is the main cause of erosion, for it makes the surface soil hard and as impermeable as concrete, so that the water rushes off and the lower layers remain dry as in a desert. Goats have caused much erosion in Mediterranean countries, but not in tropical Africa; when cattle are driven away from a place through the spread of tsetse fly, and only sheep and goats can be kept, the grass cover will improve; sheep and goats graze more closely than cattle, but they tread more lightly.

Agriculture in temperate regions is changing rapidly, and so is the attitude to change; it is welcomed. The outlook towards African agriculture is changing; there used to be elaborate statistical experiments to give proof of small differences of yields per acre, under conditions

where it was labour and not land which was the limiting factor; many panaceas have been recommended during the past thirty years—green manure crops, contour ploughing and mixed farming are some of them. But an increasing number of agricultural scientists are now demonstrating the wisdom of African farmers, of mixed planting to cover the soil and of giving a minimum of cultivation. We used to be taught that cultivation was necessary; my belief in it was shattered by an old Muganda's explanation of the vigour of a magnificent spreading coffee tree outside his house: "I sweep the ground every day".

Ecology is changing; as Raup showed in his address to the Jubilee Meeting of the British Ecological Society, Clementian ideas of plant succession have been much modified or discarded in America[4]. The influence of these ideas, alas, has extended to Africa. "Ecological studies in the tropics and sub-tropics have been dogged by attempts to fit the observed facts into a framework of classification tracing back to the ideas evolved in temperate Europe and North America, usually those of the schools of Cowles–Clements–Tansley or the Zurich–Montpellier group. The results are often worse than useless; they are misleading, and he who wants a knowledge of the ecological units present in a region has to disentangle factual descriptions from abstract discussion of successional relationships."[5]

The travellers who went to Africa in the nineteenth century had no abstract ecological concepts; they described what they saw, and their writings seem factual, all except those of Stanley, who was a journalist and wrote for effect; if he had known those concepts, he would have used them even more than do some journalists at the present day. Truth of the other accounts has been checked by careful examination on the ground and has shown some astonishing changes; for example, there is no doubt that one hundred years ago there were in Uganda far more people and far fewer trees than at present.

There have been many modern maps of Africa which were compiled by people much influenced by theories and philosophies, based on concepts which cannot be grasped by those who doubt their validity. Even the word 'ecosystem' can have no precise meaning in Africa, where mammals migrate for hundreds of miles and birds for thousands of miles; no region is free from influence outside it; there has been much outcry about the advance of the Sahara, but little mention of the great benefit from the vast quantities of base-rich desert dust brought down by the harmattan wind.

Changes are rapid in Africa. I recently took back to Africa some of the photographs which I had made about thirty years before; they proved changes which I had not expected. Cameras have no ecological concepts, they record facts; and the facts, not the concepts, will be useful in the future.

There is a great danger that, with increasing education, much of the traditional lore of African peoples will be lost, that Africans will place too much trust in the written word. It took me years to work out that the only feasible explanation for localized patches of reddened soil was that they were sites of settlements; an African assistant said: "You are quite right, sir; our people have always known that", and gave instances which I had not noticed. There continue to be many theories about the origin of lateritic ironstone from natural causes, but little attention to the frequency of worked stones, even of grindstones, in it.

Tribal history may be more significant than any survey in the explanation of existing vegetation. There were many wars between Ashanti and the Ivory Coast, so the forest on the borders is young, growing over former farmland. The Mabira Forest by the River Nile, on the borders of Buganda and Busoga, is tall, but it also is young; soil tests for a tea planter indicated a house site in an unlikely spot; a fortnight later, the headman who was clearing the land greeted me: "You said truly, sir; when we dug where you said the house had been, we found this, and I kept it to show you". It was a hand-made hoe, the most treasured possession of a farmer and buried in the floor of his house; he had been killed or enslaved, the house had disappeared; only the hoe was left, sealed in the clay; and it was not even rusted.

Much of Africa has been photographed from the air to give a true picture of the general aspect. If this photography is combined with objective recording along transects and not by subjective surveys, then a factual and more detailed description is obtained. Specimens can be correctly identified by reference to herbaria like Kew, where floras of the African regions are being compiled. There is need for some agreed system of recording, so that the results for one country can be compared with those of another; and there is need for simple descriptive words like woodland, instead of imported vernacular terms like savanna, so incorrectly used sometimes in Africa.

Africans are skilled at recognizing plants from their leaves, which must be done in plant recording, for there is no season when all species will be in flower or fruit. Moreover, Africans must not be subjected to ecological theories before they do this recording; so many concepts have been enunciated, accepted, disproved and discarded during the short history of the subject that those at present in fashion are unlikely to last. As I had no training in ecology, I was not exposed to the full impact of the obsolete ideas in vogue when I was a student; but I had much training in soil science and was so indoctrinated with soil erosion that I had been years in Africa before I clearly understood that the grey soils in the valleys were poorer than the red soils on the hills—a pattern which is common in East and West Africa.

Tribal history and legends should be recorded and investigated before they are lost; they may be of more significance than some philosophies, for they do have some connexion with fact. Their examination will facilitate true knowledge of the past, the present and the possible future; for example, the Hamitic people and their cattle moved across Uganda from the north-east about four hundred years ago, which would not be possible at the present time; there must have been open grassland then, with fewer trees, tsetse flies and trypanosomes than there are now. More history and less philosophy are needed for progress in ecological studies in Africa (and, for that matter, in Britain as well). Furthermore, it is far better to leave blank spaces in maps than to fill them in with generalized inferences.

The spectacular increases in yields of cocoa which have followed scientific use of insecticides and fertilizers show that production in Africa need not be limited by 'natural' resources, as is assumed by those whose proposals are limited by ideas of 'conservation'. If there was less abstract discussion, if agriculture was left to the agricultural scientists and farmers, if forestry was left to the foresters, then the conservationists could devote their attention to the problems of wild life, so urgent in Africa. There is more than enough for them to do if the term 'conservation' was altered to 'wild life management' as in America; for the state of some Nature reserves is alarming —the destruction of grass by hippopotamus in the Queen Elizabeth National Park of Uganda and the extermination of trees by elephants in the Murchison Falls National Park.

Africans have adopted from other continents the things which they liked, such as bananas for food, cocoa for cash and football for recreation; they have rejected the things which they did not like, such as some of the labour-wasting methods of farming which have been advocated. Some African practices may seem strange to new-comers, but there is a reason, or there has been a reason, for them. It must not be expected that the

evolution of cultural change will be exactly the same in Africa as in other continents. Those who go to Africa must consider it their duty to learn before they start to teach. "There is all Africa, and her prodigies in us."[9]

[1] Thomas, A. S., *J. Ecol.*, **33**, 10, 153 (1946).
[2] Brammer, H., *Agriculture and Land Use in Ghana*, edit. by Willis, J. B., 89 (Oxford Univ. Press, 1962).
[3] Buxton, P. A., *J. Ecol.*, **23**, 134 (1935).
[4] Raup, H. M., *British Ecol. Soc. Jubilee Symp.*, 19 (1964).
[5] Coldrake, J. E., "Some Concepts and Methods in Subtropical Pasture Research", in *Bull.* 47, *Com. Bur. Pastures and Field Crops* (1964).

NEWS and VIEWS

Deputy Chief Scientific Adviser, Ministry of Defence : Prof. A. H. Cottrell, F.R.S.

On June 30, Prof. A. H. Cottrell left the Department of Metallurgy at Cambridge to take up the post of Deputy Chief Scientific Adviser to the Ministry of Defence. This represents the beginning of a new phase in the career of an outstanding scientist who has already made great contributions in the academic and public sectors of science. He was professor of physical metallurgy at the University of Birmingham from 1949 until 1955 and was then appointed deputy head of the Metallurgy Division of the Atomic Energy Research Establishment at Harwell. From there he went to Cambridge in 1958 as Goldsmiths' professor of metallurgy. The interchanges in his career between academic and applied science environments are representative of Prof. Cottrell's attitude to his work. Although he is a first-rate theoretician, he nevertheless has a deep-rooted belief in the importance of applied science. This is revealed in much of his scientific writing, in which theories are developed not as an end in themselves, but as a means of solving the problems which arise when real materials are used in practice. His gift of picking out the essential features of a problem makes his papers and books stimulating reading for first-year undergraduates and research workers alike, and the same quality is evident in his lectures. He was elected a Fellow of the Royal Society in 1955 and, although still only in his middle forties, he has been honoured by many scientific bodies and universities at home and abroad. While at Cambridge he devoted himself wholeheartedly to both teaching and research, and the results will continue to benefit the Department of Metallurgy for many years to come. He will be greatly missed, but Cambridge's loss is the nation's gain since it would indeed be difficult to imagine anyone better equipped to deal with the responsibilities of his new post.

Ministry of Aviation Appointments: Mr. H. G. R. Robinson, O.B.E.

Mr. H. G. R. Robinson has been promoted to deputy chief scientific officer and appointed head of the Instruments and Electrical Engineering Department at the Royal Aircraft Establishment, Farnborough. Mr. Robinson joined the Guided Weapons Department of the Royal Aircraft Establishment in 1948 after graduating with honours in electrical engineering at the Imperial College of Science and Technology. After a year of postgraduate study at California Institute of Technology in 1952, he rejoined the Guided Weapons Department where he was engaged in theoretical and free flight investigation of kinetic heating at very high speeds. This work led, in 1955, to the design of the *Black Knight* re-entry test vehicle, with which Mr. Robinson was directly concerned. In 1960 he became head of the Satellite Launcher Division of Space Department, Royal Aircraft Establishment, and continued in this Department until his present appointment. His main concern in the Space Department was the British technical contribution to the European Launcher Development Organization (E.L.D.O.) launcher *Europa* I, and development of *Black Knight*, for space applications. Mr. Robinson is a Whitworth Scholar, and was awarded the Bronze Medal of the Royal Aeronautical Society in 1960 for furtherance of ballistic missile techniques.

Mr. R. R. Duddy

Mr. R. R. Duddy has been promoted to deputy chief scientific officer and appointed director of the *Concord* Project in the Ministry of Aviation. Mr. Duddy was educated at Judd School, Tonbridge, and took a first-class honours degree in mechanical engineering at King's College, London. He first joined the Royal Aircraft Establishment in July 1939 as a vacation student in the Aerodynamics Department, and from 1954 until 1959 he was head of the Flight Division of the Department. He then spent two years with the Defence Research Staff in Washington and returned in 1961 to become head of the Naval Air Department of the Royal Aircraft Establishment at Bedford.

Mathematics in the University of Edinburgh: Prof. F. F. Bonsall

Prof. F. F. Bonsall, at present professor of pure mathematics in the University of Newcastle upon Tyne, has been appointed to the McLaurin chair of mathematics in the University of Edinburgh as from October 1. Prof. Bonsall was educated at Merton College, Oxford. In 1947, after an undergraduate career which had been interrupted by six years of wartime service in the Royal Engineers, he graduated with first-class honours, and was appointed to a lectureship in mathematics in the University of Edinburgh. He joined the Department of Mathematics in Newcastle as a lecturer in 1948, was promoted to a readership in 1956, and has held the chair of pure mathematics at Newcastle since 1959. Prof. Bonsall has made substantial original contributions in functional analysis. His early work in this field dealt with some of the more algebraic aspects of the theory of Banach algebras. He then became interested in partially ordered vector spaces, a subject on which he is a leading authority. Generalizing a result of Krein and Rutman, he proved that various classes of positive operators possess positive eigenvectors. His work in this area also includes the study of order ideals, related extension theorems for linear functionals, and numerous applications in other branches of analysis. His recent researches deal with the classification of certain semi-algebras of functions, the structure of locally compact semi-algebras, and the further investigation of the spectral properties of positive operators by means of semi-algebra theory.

Industrial Engineering in the University College of Swansea : Dr. T. O. Jeffries

Dr. T. O. Jeffries, who has recently been appointed professor of industrial engineering in the University College of Swansea, was educated originally as a physicist, having gained an honours degree in physics from New College, Oxford, in 1947, with a special subject in nuclear physics in 1948. After this he was for three years Faraday Fellow at St. John's College, Oxford, during which time, in addition to his tutorial and lecture duties, he was concerned with the borderline between physics and engineering, participating in the design and construction of the Clarendon Laboratory 400-keV and 1-MeV Cockcroft and Walton accelerators, and completely designing and constructing a smaller 100-keV accelerator to study the D–D reaction. From the Clarendon, Dr. Jeffries moved

to the University of Birmingham as lecturer in physics, where among other work he designed and constructed a propane bubble chamber for use with the 100-MeV proton synchrotron. In 1956 he joined the Atomic Power Division of the English Electric Co., where he worked successively as physicist, mathematician and finally as head of the control and instrumentation group, a department of about 140 engineers, scientists, draughtsmen and technicians. While with the English Electric Co., Dr. Jeffries was responsible for designing and building the *Saturn* analogue computer and the smaller *Mars* computer, as well as for the design and construction of a *Monte Carlo* analogue computer which he used for the shielding calculations on nuclear reactors. During this period, Dr. Jeffries became extremely interested in the application of computers to design problems and to the use of data processing techniques to assist with the design of circuits and in the production of wiring and cabling diagrams.

Inorganic Chemistry in the University of Aberdeen : Prof. H. F. W. Taylor

Dr. H. F. W. Taylor took up, on October 1, his appointment as the third professor in the Department of Chemistry of the University of Aberdeen, with special responsibility for inorganic chemistry. Taylor, who hails from Nottingham, studied at University College there, graduating B.Sc. (London) with first-class honours in chemistry; he worked with the late Prof. J. M. Gulland and Dr. (now Prof.) D. O. Jordan on the physical chemistry of nucleic acids—work for which he was awarded a Ph.D. (London) in 1947. On leaving Nottingham he carried out research at Bedford College under Dr. (now Prof.) R. M. Barrer (hydrothermal chemistry of alumino-silicates) and at Birkbeck College under Prof. J. D. Bernal (chemical and crystallographic aspects of the hydration of Portland cement and related topics). For these and later contributions to the chemistry and crystallography of silicates Taylor was awarded the degree of D.Sc. by the University of London in 1957. When appointed lecturer at Aberdeen in 1953 (senior lecturer, 1963) Dr. Taylor continued his studies into the wide field of silicate chemistry especially by the use of X-ray techniques, and he has built up, assisted by Dr. F. Glasser, an active and enthusiastic research team. Investigations are being carried out along two main lines, calcium silicate hydrates and the chemistry of cement hydration—work which gained for him the Mineralogical Society of America Award for 1959, and also on topotactic reactions. His work in these fields is of international repute and he has been invited to take part in and has helped to organize conferences, etc., in Britain, the United States and elsewhere. He is the author of some eighty papers, editor and part author of *Chemistry and Cements*, and co-author with L. Heller of *Crystallographic Data for Calcium Silicates*. Following in the tradition of the Scottish universities, Dr. Taylor has taken, and will continue to take, a great interest in the teaching of the first-year classes. He has found this a challenging problem in both content and method and has introduced, into the practical course, programmed learning, multiple choice testing and the use of the computer for certain types of marking.

Chemistry in the University of Reading

The years 1965 and 1966 form an important period in the development of the Department of Chemistry in the University of Reading. Now established in one of the largest of the new buildings in Whiteknights Park, the Department will at the end of the session 1965–66 lose the services of its present head, Prof. E. A. Guggenheim, who was appointed professor of chemistry in 1946, having been a member of the Department since 1933. An appointment to a second chair in the Department has already been made: Prof. D. Bryce-Smith, professor of organic chemistry, took up that post in June.

The appointment is now announced of professors in physical and in inorganic chemistry. Dr. G. W. Fowles, who will become professor of inorganic chemistry from January 1, 1966, and Dr. H. M. Frey, who will become professor of physical chemistry from October 1, 1966, are both at present on the staff of the University of Southampton.

Prof. G. W. Fowles

During 1943–44 and 1945–46, Dr. Fowles was an analyst in an industrial laboratory, spending the intervening period in H.M. Forces. He entered the University of Bristol in 1946, and in 1949 obtained the degree of B.Sc. with first-class honours in chemistry. He then continued with research and gained his Ph.D. in 1952. Dr. Fowles was awarded the D.Sc. degree in 1964. He was appointed to the Department of Chemistry in the University of Southampton in 1952, where he is now reader in inorganic chemistry. He was Canadian Research Council–Nuffield Foundation visiting professor for 1959 at Laval University, Quebec. He has twice visited the United States, for a Gordon Research Conference and a meeting of the American Chemical Society; and he has spoken at the seventh (1962) and eighth (1964) International Conferences on Co-ordination Compounds. During 1962–65 he was local representative and a member of the Council of the Chemical Society. His research has been concerned mainly with the chemistry of transition metals of the first part of the Periodic Table, although he is also actively working on organometallic compounds of other metals. His present research group is principally concerned with the spectroscopic and magnetic properties of co-ordination compounds of elements of the titanium, vanadium, and chromium sub-groups. He has published numerous papers, and, with E. Cartmell, a book, *Valency and Molecular Structure* (Butterworths, 1956 and 1961).

Prof. H. M. Frey

Dr. Frey, after National Service in the Royal Air Force, entered Balliol College, Oxford, in 1949, and in 1951 was University Gibbs scholar in chemistry. He obtained first-class honours in chemistry in 1953, and then became War Memorial student and Imperial Chemical Industries Research Fellow, gaining his D.Phil. in 1955. During 1955–57 he held a Commonwealth Fund Fellowship at the University of California at Berkeley and at Harvard University. In 1957 he was appointed lecturer in the University of Southampton, and has been senior lecturer since 1963. His principal research has been concerned with the elucidation of the features and mechanism of inter- and intra-molecular energy transfer, particularly in relation to unimolecular reactions. Virtually all this work has been in the gas phase, though at present some investigations are being extended to the liquid phase. Much of his recent work has been on highly vibrationally excited molecules formed by methylene addition reactions, or by the photochemical decomposition of diazocompounds and diazirines.

Microbiology in the University of Sussex : Prof. J. R. Postgate

Dr. J. R. Postgate, who has been appointed to a chair of microbiology in the University of Sussex, was educated at Kingsbury County School and Balliol College, Oxford, which he entered as a Williams exhibitioner in 1941. He graduated in chemistry with honours in 1945, and found his interest in microbiology when taking his Part II in the field of bacterial growth kinetics under Prof. (now Sir) Cyril Hinshelwood. This interest was developed by a year's study of microbial chemistry under the combined tutorship of the late Prof. D. D. Woods and Prof. A. G. Ogston, and his transformation from chemist to biologist was complete when he took his degree of Ph.D. on bacterial drug resistance under Prof. Woods in 1948. He then joined K. R. Butlin's group

at the Chemical Research Laboratory, Teddington, where he carried out his well-known work on sulphate-reducing bacteria and associated problems in economic microbiology. This work was notable for the discovery of cytochrome c_3, the first cytochrome to be found in an anaerobic organism, and for developing techniques for handling and studying sulphate-reducing bacteria. This very successful phase of Dr. Postgate's work was abruptly closed by the disbandment of K. R. Butlin's group in 1959, and Dr. Postgate then undertook and published research on the death of bacteria in conditions of freezing and starvation at the Microbiological Research Establishment, Porton. There he remained until 1963, with an intermission as visiting professor of microbiology in the University of Illinois during 1962–63. In 1963 he joined the Agricultural Research Council to assist Prof. J. Chatt to establish a new Unit of Nitrogen Fixation, now located at the University of Sussex. He will continue to be assistant director of the Unit and his chair is a personal one for the period of his association with the Unit.

Psychology in the University of Newcastle upon Tyne: Prof. J. Brown

Dr. J. Brown has been appointed to the chair of psychology in the University of Newcastle upon Tyne with effect from August 1, 1966. Dr. Brown was born in August 1925. He went to Emmanuel College, Cambridge, graduating B.A. in 1950 after reading the Moral Sciences Tripos. He gained his Ph.D. degree in 1955, and spent three years on research at Cambridge after graduating and then spent one year as a temporary assistant lecturer in the University of Bristol. In 1954 he went to Birkbeck College as assistant lecturer, becoming lecturer in 1956. For the year 1965–66 he has been appointed visiting associate professor in the University of California (Berkeley). His main interests are in perception, learning and remembering, especially problems of immediate memory.

United Nations Review of Space Science

The United Nations Committee on the Peaceful Uses of Outer Space was formed in 1959, and has served a useful function as the only international governmental body concerned solely with the subject. In 1963 the Committee decided to sponsor a publication describing the activities of the various international and national organizations concerned with space, and this report has now been published by the United Nations under the title *Space Activities and Resources* (Pp. v+172. (*A/AC.* 105/26.) New York: United Nations, 1965. 2 dollars). The report covers first the international governmental organizations which are in some way concerned with space, such as the International Telecommunication Union and the World Meteorological Organization. The next sections describe regional organizations, such as the European Space Research Organization, and non-governmental bodies such as the Committee on Space Research (COSPAR) of the International Council of Scientific Unions. The remainder of the report, about two-thirds of the whole, reviews national space activities. Although the United States and the U.S.S.R. have the largest contributions, there are reports from a total of 37 countries, ranging from Argentina to Yugoslavia, and covering the period up to early 1964. This publication will be welcomed as a work of reference by anyone wishing to sort out the tangled inter-relations between the many international organizations which have decided that space is a subject they cannot afford to ignore. It also provides a useful review of national space activities, especially those of the smaller countries, which are little publicized.

Molecular Pharmacology

The first reaction of a scientific reader to a new journal is one of alarm: he has already to look at too many of them and his absorptive capacity is limited. Moreover, the title of the recently issued *Molecular Pharmacology* is really a tautology. Pharmacology is the science of the examination of the interaction of organic and inorganic molecules with living matter. It follows that all pharmacology is molecular. However, much can be said in defence of this new journal (1, No. 1, July, 1965. Edited by Avram Goldstein. Pp. 1–112. New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965. Subscription rates: Vol. 1, 1965, 3 issues—International subscribers, 11 dollars; Private subscribers certifying that the journal is for personal use only, 5 dollars). It is being launched under the auspices of the American Society for Pharmacology and Experimental Therapeutics, which is also responsible for the well-known *Journal of Pharmacology and Experimental Therapeutics*, a publication that carries much material of immediate interest also to the physiologist and clinician. The need may have been felt for a paper that appeals more to the reader whose interests lie mainly in the theoretical aspects of pharmacology. A glance at the first issue shows that it is mainly the development of modern biochemistry (and biophysics) that has made this new venture necessary. The study of enzymes, of proteins in general, of nucleic acids, of cell particles and of cell membranes makes up the main content, and there is also material that deals with transport, with receptor theory and with the genetic code. *Molecular Pharmacology* carries the sub-title "An International Journal". The members of the advisory board and of the editorial board include scientists from many lands, well known to all workers in this field. The editor is Dr. Avram Goldstein, of Stanford University Medical School. We wish him success in this new enterprise.

Use of CIBA Resins in Ireland

The growth potential in the use of synthetic resins and ancillary products in rapidly expanding industries both in Ulster and in the Republic of Ireland has been recognized, since the beginning of 1965, by the establishment by Ciba (A.R.L.), Ltd., Duxford, Cambridge, of an office and distributing depot in Belfast. Some details of actual developments involving the use of the well-known 'Aerolite' and 'Araldite' products in blockboard and paint manufactures, for example, are given in a recent issue of *Technical Notes*, entitled "Ciba Resins in Ireland" (Pp. 8. Ciba (A.R.L.), Ltd., Duxford, Cambridge. June 1965). In Northern Ireland there has been, in recent years, a notable expansion in the building industry, especially concerning prefabricated units. One example is the manufacture of wood chipboard, involving the use of large quantities of 'Aerolite' resin each week, at the factory of the Ulster Chipboard Company at Coleraine, County Londonderry; in this case the raw material is forest thinnings from timber felled from three of the largest forests in Northern Ireland; the process consists of coating the wood chips with resin, pressing the boards, then sanding on both sides to specified thickness. Other firms using 'Aerolite' adhesive are in large-scale production of such items as panel doors, flush doors, casement windows, garage doors, staircases, school and church furniture. In Eire, similar activities and modern trends in prefabricated timbers are briefly described. In the factories of T. and C. Martin, Ltd., Dublin, a finger-jointing machine produces continuous lengths of jointed timber by gluing selected raw timber of relatively short length with 'Aerodux 500' resorcinol resin adhesive, clamping and pressing, then cutting automatically to the desired length; timbers jointed in this way are eminently suitable as laminated members for structural engineering. Among paint manufacturers, Syntheses, Ltd., Dublin, use 'Araldite' epoxy resin formulations in their production of high-duty paints demanding good mechanical and chemical resistant properties. A further example of this is quoted in the case of Kosangas, Ltd., Dublin, suppliers of

gases in steel bottles—containers which are subject to hard use; they are successfully coated with 'Araldite'-based paint, after shot-blasting, applied by electrostatic spray. These Ciba resins are being increasingly used by other industries in Ireland and such uses are planned for description in forthcoming issues of *Technical Notes*.

Iron Ore Production and Research at Broken Hill, Australia

The well-known *B.H.P. Technical Bulletin*, produced periodically by the Broken Hill Proprietary Co., Ltd., Australia, is always a source of valuable technical information on the iron ore industry, arising from operational and research activities within this great organization, and the issue published in April 1965 is no exception to this dictum (No. 22: 9, No. 1; April 1965). An important contribution is a series of articles describing a new ore agglomeration laboratory built at the Company's Central Research Laboratories at Shortland, New South Wales. A general description of the new premises is first given by L. C. Bogan, who describes the laboratory layout, technological capabilities and limitations of the pilot plants installed, programmes scheduled, and services available; the latter includes chemical analysis by direct-reading spectrophotometry, petrology, and X-ray diffraction and X-ray spectroscopy. "Comminution and Green Pellet Production" is the title of a second article, by D. H. Harraway, in which he discusses the several pilot plant operations involved in production of green pellet agglomerates, comminution of the ore and mixing it with additives and water to form the ore pellets. A third article, "The Experimental Pellet Heat-Hardening Unit", by C. W. Brock, describes how, having produced the green pellets, it is necessary to heat-treat them ". . . to form a strong, approximately spherical aggregate capable of withstanding weathering and the high handling stresses which may be expected in stockpiling, transportation and blast furnace charging". Further contributions to this *Bulletin* include an account of "The Development of Koolan Island", by M. T. Phillips; this is one of the islands of the Yampi group off the north-west coast of Australia, where production of iron ore commenced in 1964. Petrological research is represented by an interesting paper by Sylvia Whitehead entitled "Petrological Notes on the Koolyanobbing Iron Ore Formation", the location being the Koolyanobbing range of hills situated about 35 miles north-east of Southern Cross, Western Australia. An important haematite ore-producing region in South Australia is the Middleback Ranges, and K. L. Ashworth describes this and other prospects in his article on "Geology of Iron Duke, South Australia", situated approximately 36 miles west of Whyalla, on the eastern Eyre peninsula. Finally, there is a paper by J. A. Gregory and S. Wolski on "Experimental Assessment of Factors Controlling the Production of High-Basicity Sinter".

South African Prehistory

An interesting pamphlet entitled *Bowmen, Spears and Shields in Southern Rhodesian Rock Art*, concerned with the types of shield depicted by the artists and the distribution of the bowmen and the spears, has recently been published (*Cimbebasia—SWA Research*. No. 10. Pp. 8. Windhoek, S.W.A.: State Museum, 1964). The author, C. K. Cooke, has personally visited more than a thousand rock shelter and cave sites and has, of course, drawn on the works of other authorities. The important fact which emerges is that the shields figured are of definitely Bantu type, and this would suggest that many of the painted rock shelters must be A.D. in date. It has been the custom to ascribe a great antiquity to the rock paintings of Southern Rhodesia and South Africa. However, it has constantly been forgotten that paintings in open rock shelters could scarcely have survived serious climatic changes, such as have occurred in the distant past —conditions which permitted much precipitation and subsequent evaporation of water vapour on the rock shelter walls. It is therefore very interesting that research into the types of shield figured in this late phase of Southern Rhodesian rock art points to a comparatively late date for its creation. The late C. van Riet Lowe once remarked that the celebrated "White Lady" of Brandberg could as well have been painted in A.D. 1500 as in 1500 B.C.! The June issue of the *South African Journal of Science* contains an interesting article by R. R. Inskeep. Not far from Port Elizabeth a series of springs on the hillside behind the homestead on the Amanzi Estates has deposited thick sediments which contain many lower palaeolithic implements. The author has undertaken some considerable excavation and gives us geological sections and a determined stratigraphy. The implements are almost all of the core type, though some worked flakes do occur. Hand-axes, cleavers and choppers have been collected, and the author dates the finds to an early stage in the Old Stone Age. Some samples of pollen were noted, and tentative identifications indicate the presence of members of the Cyperaceae, Gramineae, Compositae and Leguminosae (including an acacia) families. Mr. Inskeep is to be congratulated on a useful piece of work.

Bark Canoes and Skin Boats of North America

Like many traditional crafts, the building of bark canoes is dying out and much information about it has already been lost. E. T. Adney, who died in 1950, was a skilled artist, who devoted a good deal of his life to the study of these canoes and acquired practical experience of building them. He left a lot of models, now in the Mariner's Museum at Newport News, Virginia, as well as numerous papers "in a highly chaotic state". H. I. Chapelle, who is curator of transportation in the Museum of History and Technology, a constituent of the U.S. National Museum, has carried out the arduous task of compiling the relevant information from these papers and the models (Smithsonian Institution, Washington. Museum of History and Technology. Bulletin No. 230: *The Bark Canoes and Skin Boats of North America*. Pp. xiv + 242. Washington, D.C.: Government Printing Office, 1964. 3.25 dollars). He has added a chapter on Arctic skin boats and one on temporary craft—canoes of bark other than birch, moose-hide boats and the coracle-like bull boats. There is an appendix on rolling kayaks by J. D. Heath. Information derived from Adney makes up the main part of the book. It consists of three chapters on early history, materials and tools, and form and methods of construction, followed by three chapters treating in detail the canoes of the Eastern Maritime Region, Central Canada, and North-West Canada, of which the first is the fullest. The work is fully illustrated with photographs from many sources and excellent drawings, mostly redrawn from Adney's by Chappelle. Amid a wealth of detailed information a fact of general interest emerges. There is a fundamental difference between bark canoes and Eskimo skin boats, namely that the frame of the bark canoe (and this includes 'temporary' Indian skin boats) is built up within the cover and would collapse without it, whereas the Eskimo boats have a rigid frame over which the skin cover is afterwards stretched. As is well known, bark canoes are so suitable for their purpose that they were at once adopted by Europeans on arrival, and used on a large scale in the fur trade. This suggests that this valuable publication may find a practical use in addition to its great historical importance.

Production Ecology in Tropical High Forest

Not much work has been carried out on production ecology in tropical high forest although it is estimated that 38 per cent of the forests of the world belong to this type. However, a very useful investigation has been carried out in the southern Ivory Coast (*Production*

Brute, Pertes par Respiration et Production Nette dans la Forêt Ombrophile Tropicale. Par D. Müller et Jörgen Nielsen. Pp. 69–160, illus. *Det. Forstlige Forsøgsvaesen I.* Danmark, 1965). Field measurements were made in 1955, 1957 and 1960, in two sample plots measuring 30 m × 30 m and 40 m × 40 m. Altogether there were 209 trees of more than 3 cm diameter breast height but 58 per cent belonged to 5 of the 45 species represented. All the trees in the smaller plot were felled after the 1955 measurements, care being taken first to clean a sufficient area so that the entire crowns fell on places where it was possible to collect all the leaves from the sample trees. In the larger plot basal areas, form factors, volumes and current annual increments were calculated from diameter and height measurements. Other data provided statistics for the branch percentage, loss of dry matter by respiration, surface area and weight of leaves, leaf-fall and photosynthetic efficiency. Special attention is given to the occurrence of 'light' leaves and 'shade' leaves which is a characteristic shared by deciduous trees of temperate and tropical forests, but dominants in the latter tend to have 'light' leaves only and it is suggested that this is why their crowns are usually small. In this particular example the net production of dry matter came to 9·0 metric tons per hectare per annum (roots, stems and branches) but the gross production was 52·5 metric tons per hectare per annum. It is interesting to note that although the production of dry matter is only very slightly less than for an even-aged beech forest in Denmark, the gross production is more than twice. Indeed, it is a characteristic of lowland tropical high forest that there is a large gross production and a great loss of dry matter by respiration in the stem, branches and leaves. This is a most welcome contribution to the subject of production ecology and the authors have provided useful data. Perhaps the exercise also demonstrates the difficulty of taking an adequate sample in tropical high forest and yet keeping the work within the realm of practical possibility. For example, familiar dominants of the tropical high forest of West Africa are absent from the tree lists given. In the smaller plot, the second largest tree by diameter (63·6 cm) and in height (27 m) is *Turraeanthus africanus*, which is not a typical emergent of this forest. The tallest tree recorded from both plots is 50·2 m and only two others reach 40 m or more.

Elementary Particle Physics

Following the review of *Group Theoretical Concepts and Methods in Elementary Particle Physics* (*Nature*, **207**, 118; 1965), Messrs. Gordon and Breach, Science Publishers, Inc., have written to the Editor, stating that two prices are available for this book. There is a professional edition published at 14.50 dollars, and a reference edition published at 21.50 dollars.

Announcements

The Wellcome Laboratories of Tropical Medicine which since 1934 have been housed in the Wellcome Building, Euston Road, London, have moved to new laboratories on the site of the Wellcome Research Laboratories at Langley Court, Beckenham, Kent.

With the July 1965 issue, the *Bulletin of the Libraries of Commerce, Science and Technology* of the Sheffield City Libraries takes a new style and arrangement (No. 1, July 1965. Pp. 18. Sheffield: Sheffield City Libraries, 1965). While continuing to notify accessions, with the exception of some pamphlets and miscellaneous material, the *Bulletin* will now announce any developments within the libraries affecting readers and users of their services. The present issue includes a list of directories and also a list of recent metallurgical reports.

Open days will be held at the Tropical Products Institute during October 14–15. Further information can be obtained from the Director, Tropical Products Institute, 56–62 Gray's Inn Road, London, W.C.1.

A conference on "Technology and Society" will be held in Bath during October 14–16. Further information can be obtained from Mr. J. H. Lamble, Bristol College of Science and Technology, Ashley Down, Bristol 7.

The third INTERKAMA Exhibition and Congress will be held in Düsseldorf during October 13–19. Further information can be obtained from Nordwestdeutsche Ausstellungsund Messe-Gesellschaft m.b.H., 4 Düsseldorf 10, Postfach 10203.

The Arthur Stanley Eddington Memorial Lecture for 1965, entitled "The Brain and the Unity of Conscious Experience", will be delivered by Sir John Eccles in Cambridge on October 15. Further information can be obtained from W. B. Harland, Sedgwick Museum, Cambridge.

A symposium on "The Future Pattern of Research in Instrument Technology", arranged by the Tees-Side Section of the Society of Instrument Technology, will be held at Middlesbrough on October 14. Further information can be obtained from Mr. G. F. Shute, 10 Mayberry Grove, Linthorpe, Middlesbrough, Yorkshire.

The ninth conference on "Analytical Chemistry in Nuclear Technology", sponsored by the Analytical Chemistry Division of the Oak Ridge National Laboratory, will be held in Gatlinburg, Tennessee, during October 12–14. Further information can be obtained from C. D. Susano, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37831.

An international conference on "Dynamic Stability of Structures", sponsored jointly by the U.S. Air Force Office of Scientific Research and Northwestern University, will be held in Evanston, Illinois, during October 18–20. Further information can be obtained from Prof. George Herrmann, Technological Institute, Northwestern University, Evanston, Illinois.

The annual meeting of the Conference on Electrical Insulation of the National Academy of Sciences–National Research Council will be held at Buck Hill Falls, Pennsylvania, during October 25–27. Further information can be obtained from Mr. D. W. Thornhill, Conference on Electrical Insulation, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C.

Corrigendum. In the article entitled "Environmental Determination of Insular Variation in Bird Species Abundance in the Gulf of Guinea" by Prof. T. H. Hamilton and N. E. Armstrong, which appeared on p. 148 of the July 10, 1965, issue of *Nature*, the following corrections should be made: (1) Second paragraph, line after equation (4) should read: "where $z \neq 1$" (not '$x \neq 1$'). (2) Third paragraph, thirteenth line, should read: "estimate the radius . . . as 7·25 miles" (not '. . . as 7 . . .'). (3) Third paragraph, seventeenth line, should read:

"$\ldots \pi r\sqrt{r^2 + h^2}$" (not '$\pi r\sqrt{r^2 - h^2}$ ').

Corrigendum. In the communication entitled "Radiochemical Determination of the Endogenous and Exogenous Respiration of Bacterial Spores" by H. Desser and Prof. E. Broda, which appeared on p. 1270 of the June 19, 1965, issue of *Nature*, line 33 should read ". . . The specific activities were up to $6{\cdot}6 \times 10^3$". Also, H. Desser and Prof. E. Broda thank Prof. H. O. Halvorson, Madison, for the gift of *Bacillus cereus* and for advice on its cultivation.

Erratum. In the communication entitled "Benzenesulphonylcarbamates: New Herbicides" by Helen J. Cottrell and Dr. B. J. Heywood, which was published on p. 655 of the August 7, 1965, issue of *Nature*, the first reference should read as follows:

[1] Carpenter, K., Heywood, B. J., Parnell, E. W., Boesch, R., and Metivier, J., Brit. Pat. Appln. 32407/61, and Brit. Pat. Appln. 8805/63.

FUEL POLICY FOR BRITAIN

On January 22, 1965, Mr. F. Lee, the Minister of Power, told the House of Commons that he was undertaking a general review of fuel policy and had already initiated discussions with the chairman of the nationalized fuel industries. To assist consultation on a wider basis he was setting up an Energy Advisory Council. This Council would consider and advise him about the energy situation and outlook, and about plans and policies of the fuel and power industries in relation to national objectives for economic growth. At the end of June he told the British Electrical Power Convention at Brighton that he expected the results of this review of fuel policy to be ready soon. At the same time, he asked the industry to increase capacity to the point where reasonable, but not excessive margins, were secured. Moreover, he asked the industry to do this for the lowest practical demands on real resources and a very substantial use of self-generated funds, absorbing wherever possible the increases in operating costs so that prices to consumers remained stable. Concerning co-operation between the nationalized fuel and power industries in research and development, Mr. Lee said in the House of Commons on July 13 that he had already approached the industries; the National Coal Board and the Central Electricity Generating Board had for some time had a research liaison committee. The Gas Council had now joined with them to form a joint Liaison Committee for research and development so that their scientific potential might be used to the fullest advantage. The primary, though not the exclusive, objective was to improve the technology of utilizing coal. Mr. Lee said that the research programmes of the three industries were extensive, but he was bringing them into closer co-operation.

In view of all this a Broadsheet, *Questions of Fuel Policy*, issued by Political and Economic Planning*, is of some special interest. The Broadsheet starts by comparing the terms of reference of the Energy Advisory Council with the instructions given to the Ridley Committee on National Policy for the Use of Fuel and Power Resources when it was established in 1951. It points out that any recommendations the new Council may be able to agree on as to national policy are likely to represent, at the best, the highest common factor of agreement between existing vested interests. The Ridley Committee, on the other hand, was an independent group of public men, economists and technologists, examining the fuel industries rather than representing them, and was more likely to be free to formulate fresh policy in a way that the new Energy Advisory Council could not be expected to do. This in itself makes the independent review in this Broadsheet of the greater interest, although it must be admitted that in practice the advice of the Ridley Committee was not taken very seriously.

The Broadsheet notes that in 1964 Britain used primary energy equivalent to about 285 million tons of coal. Of this total about 187 million tons was actually coal; petroleum products accounted for about 93 million tons of coal equivalent, of which some 30 million tons was road- or air-transport fuel or used in refineries. Of the remaining general fuel used, coal accounted for nearly three-quarters compared with nearly 90 per cent in 1951. In absolute terms, coal consumption in 1964 was 20 million tons less than in 1951. Over the same period, the consumption of oil as a general fuel rose about five times to some 55 million tons. So far as total fuel consumption is concerned, the forecasts of the Ridley Committee in 1952 for fuel consumption during the period 1959–63 were fairly well confirmed (coal providing, however, only some 70 per cent of the total instead of 95 per cent, as forecast). Apart from the switch to oil there has been a further decline in the direct use of coal, and more and more of the final consumption of this fuel is now in the form of electricity.

On the framework of present-day policy, P.E.P. points out that imports of coal are subject to licensing and in practice no licences are granted; moreover, import licences have not been granted for any increased imports of Russian oil. Since 1961 a fairly high excise duty of almost £2 a ton has been imposed on fuel oil and other oil products sold in the general energy market, while some restraint has at times been placed on the development of electricity generation from oil. Nuclear energy constitutes another factor and the Government has also abolished the customs duty on methane, which has slightly improved the economics of the scheme for importing liquefied natural gas from Algeria.

Considering new factors in the forecasts for the consumption of fuel, the Broadsheet points out that the forecasts and investment programmes of the major fuel industries represent, even over the short term, a higher total fuel consumption than is generally forecast—as has been pointed out by the National Economic Development Council. Forecasting demand for fuels is fairly hazardous in the commercial or technical sense beyond the 7–10 year period affected by decisions regarding investment that must be taken in the next 2–3 years. Petroleum exploration in the North Sea is particularly hazardous, and however welcome might be the impact of North Sea gas to upset established forecasts, it may not be an economic proposition. A more practical factor in present calculations is the firmer offer of cheap nuclear power. The rapid downward revision of nuclear costs could mean a much higher rate of commissioning of nuclear plant in the construction programmes of the supply industry by 1975 than has so far been envisaged, and in consequence an earlier levelling off in the demand for coal by power stations.

A working group of P.E.P. which is considering the energy situation and the kind of national fuel policy most suitable for Britain in the 'sixties expects to publish a full-scale report by the autumn of 1965. In the meantime, the Broadsheet indicates some of the problems which must face those responsible for formulating policy in this field. First, it points out there is the question of whether fiscal policies, such as the levying of a fuel tax on oil used in power but not on oil used in gas manufacture, should be altered so as to favour gas less. Alternatively, should gas be assisted to take over more of the demand for space heating and electricity discouraged in this sector of the domestic market? Again, there is the question of whether Ministerial policy should attempt to settle the constantly vexed arguments between the two industries and their cross-accusations about the special financial incentives alleged to be offered to local authorities and other housing developers. Again, is the right balance between amenity and efficiency being struck at present in the lengthy procedure of licensing, public enquiry and the like required for the construction of electricity production and transmission equipment? Some might also be disposed to ask whether the present structure of Government is such as to enable amenity issues to be raised at a sufficiently early stage for full effect to be given to their arguments before the electricity authorities are committed to particular schemes. Another important question is whether research and development are adequately and properly organized in these industries,

* *Planning*, 31, No. 489 (June 1965): *Questions of Fuel Policy*. Pp. 183–218. (London: Political and Economic Planning, 1965.) 6*s*.

and in particular whether the balance of activity and initiative in nuclear development between the Central Electricity Generating Board and the Atomic Energy Authority is correct. Here again questions of Government structure may also arise, particularly in respect of the position of the Ministry of Technology.

Other questions suggested for consideration in the Broadsheet are the structural division of electricity supply for England and Wales and for Scotland, and whether there is scope for forms of combined technical development between the industries, and possibly coal or oil also, that are being ignored at present because of the structural separation and competition. The Broadsheet also questions whether limits should be put to commercially competitive promotion between the nationalized fuel industries, and how far they should be obliged to keep their investment policies with the Government's forecasts of solid and other smokeless fuel requirements arising from application of the Clean Air Policy. The principles to be followed in permitting these nationalized industries to diversify their activities in the fields outside those in which they now operate also require definition. The Broadsheet hints plainly that the consumer is not at present adequately informed of the price trends in the various fuels. The present structure of the Ministry of Power also requires consideration in the context of how far it conduces to a sensible co-ordination of national energy policy and to objective assessments of conflicts of policy between the nationalized fuel industries.

It should also be remembered that some of the technological changes that could occur in the supply of energy would bring with them new questions about the structure of the British fuel industries. For example, if large-scale natural gas-fields were in fact found in the North Sea, the present gas industry, with its virtual monopoly and its commitment to manufacture gas at lower calorific values, might not be the ideal instrument to introduce the natural gas into the British market. Possibly a measure of competition should be introduced when gas supply becomes more and more based on the end-products of oil refining. Nor should it be without due consideration whether the economies of scale in supply or in transmission are as compelling, or whether monopoly is as appropriate a form of organization for gas as for electricity supply. The Broadsheet offers no answers to these problems, but it does suggest that urgent public consideration should be given to them and particularly to the determination of the right order of priorities. This is especially so in regard to what protection the fuel industry should receive, particularly British coal, and in what form.

U.S. STANDARD FREQUENCY AND TIME SERVICES

A REVISED (1965) publication of the United States National Bureau of Standards * gives a detailed and illustrated description of the services provided by the Standard Frequency Stations now operating in Washington, Hawaii and Colorado. The first is the well-known station, *WWV*, at Greenbelt, Maryland, which has been in operation since 1933, and now provides services of radio and audio frequencies, musical pitch, time signals and the departure of these from astronomical time; propagation forecasts and geophysical alerts are also issued from *WWV*. An additional service was started from Hawaii in 1948; but this is confined to radio frequencies, time signals and their corrections. In 1963, two low-frequency transmissions were added from stations at Fort Collins, Colorado. One, *WWVB*, broadcasts continuously on the standard frequency of 60 kc/s, and provides time signals and corrections, while the other, *WWVL*, broadcasts continuously on the standard frequency of 20 kc/s. The frequencies of all these services are held stable in general to a few parts in 10^{11}, and they are ultimately referred to the resonance frequency of caesium, which is measured in terms of ephemeris time.

All the carrier and modulation frequencies at *WWV* and *WWVH* are derived from precision quartz oscillators of high stability. These are offset from the standard by a small but precisely known amount to reduce departure between the time signals as broadcast and astronomical time, U.T.2. Although the latter is subject to unpredictable changes readily noted at this level of precision, it is expected that the present offset—150 parts in 10^{10}—established in 1964, will remain in effect for the present calendar year (1965). For the benefit of users who wish to make direct comparisons of absolute frequencies, *WWVB* has been transmitting on 60 kc/s with no offset since January 1, 1965.

A comprehensive description, with diagrams, of all these services is given in the report, together with details of the propagation forecasts and geophysical alerts which are disseminated at hourly intervals. The forecast announcement tells users, in a simple code form, the condition of the ionosphere at the regular time of issue, and the quality of radio services due to propagation conditions to be expected during the next 6 h. A series of letter symbols is used to indicate the current geophysical conditions and give notice of any outstanding solar or geophysical events which are expected or which have occurred in the preceding 24 h.

The revised issue of this publication will be welcomed by all users of frequencies and time signals, as well as by research workers in the fields of radio and the geophysical and solar sciences.

R. L. Smith-Rose

* United States Department of Commerce: National Bureau of Standards. Miscellaneous Publication 236: *Standard Frequencies and Time Services of the National Bureau of Standards*. Pp. 8. (Washington, D.C.: Government Printing Office, 1965.) 15 cents.

COMMERCIAL NUCLEAR POWER STATIONS IN BRITAIN

THE principal contents of the July number of the *Journal of the British Nuclear Energy Society* (**4**, No. 3; 1965) consists of the texts of the six papers contributed by members of the Central Electricity Generating Board and the South of Scotland Electricity Board and presented to the symposium on the performance of commercial nuclear power stations in the United Kingdom, which was held at the University of Leicester on June 30.

H. M. Carruthers, in his discussion of the evolution of magnox station design, points out that, in the nine years since the first Calder Hall reactor went critical, some thirty-five reactors of this basic type have been built, of which twenty-six are in Great Britain. The reactors during this period have developed from a small unit of about 40 MW(e) output to one of nearly 600 MW(e) with at the same time reduced capital and generating costs. The main technical improvements in layout, shielding, refuelling methods, pressure circuit technology and fuel element design since the start of the commercial magnox programme are described in some detail.

The commissioning of a nuclear power station requires not only the setting of the plant to work safely and

reliably, but also at the minimum cost computed over the total life of the station. The history of the commissioning of the Berkeley and Bradwell stations is reviewed by C. D. Heath and D. J. Silverlead, and in their assessment they direct attention to the need for greater flexibility in the timing of the various tests and measurements, improvements in the planning of construction and plant installation, and some simpler form of commissioning organization.

Two papers deal with the performance of reactor cores and materials and of power station plant, and a third with power station maintenance. Of the nine civil nuclear stations, Berkeley and Bradwell have had more than two years' full-power operation and Hunterson one year. Serious defects have been experienced at all three stations and the extra fuel costs have amounted to about six and a half million pounds. The major defects were in the fuelling routes, turbines and boilers. Experience suggests that more reliable operation in the future will occur. With regard to maintenance, it is emphasized that health physics requirements are an important factor in maintenance planning and that future stations should be better planned and equipped for the maintenance of radioactive equipment.

The final paper in the symposium, by P. H. G. Holbrook and A. C. Horne, deals with some of the special problems, not necessarily technical, which arise in the operation of a nuclear power station. The provisions of the Nuclear Installations Licensing and Insurance Act, 1959, the Radioactive Substances Act, 1960, and the Generating Board's safety regulations have to be enforced and accordingly appropriate inspectorates and organizations have to be established. The increased automation of future stations, with centralized control and computational operation, may lead to a change in the pattern of station organization with economies in technical man-power and less risk to personnel.

S. Weintroub

PHYSICS EDUCATION

IDEALISM and iconoclasm were offered in equal proportions by speakers at the International Conference on the Education of Professional Physicists, held in London during July 15–21. The meeting was arranged by the Institute of Physics and the Physical Society for the International Union of Pure and Applied Physics, and attracted participants from twenty-five countries.

Lord Beeching (Imperial Chemical Industries, Ltd.), in a forthright introductory speech, deplored the attitude of university departments which, while disclaiming interest in vocational training, were in fact preparing students exclusively for the vocation of fundamental research. It was, he asserted, not in the public interest that academic research should be presented as the best career for physicists at a time when the industrial, economic and political problems of the community were increasingly dependent on the widespread use of science and technology. Though the Government had great responsibilities in the deployment of scientific and technical resources, only a handful of Junior Ministers had any scientific training. The cause of this deficiency was in the attitude which asked: "Why should we train a good physicist, only to have him wasted as a politician?"

Students of physics must learn that they were not going to be employed exclusively in the narrow practice of their specialty, Lord Beeching continued. Many graduates would find careers in which knowledge of physics was only a small part of the requirement. His own company employed 290 physicists, of whom half held posts not necessarily filled by physicists and about 10 per cent were doing work for which scientific qualifications were not essential. The object of the Government and the universities should be to create an apparent surplus of scientists, many of whom would move into management, administration and politics.

Most of the subsequent contributors were concerned with the scope and content of the undergraduate curriculum, but there was lively discussion also on the extent to which industry's needs were fulfilled by the products of the universities.

A discussion of the young physicist's training and progress in industry, offered by G. S. Bosworth (English Electric Co., Ltd.), was strongly criticized by A. B. Pippard (Cavendish Laboratory, Cambridge) as showing how second- or third-class people could be turned into useful members of an industrial team, while offering nothing to attract the first-class scientists who were badly needed in British industry but were repelled by the Philistine attitude of prospective employers.

Extreme differences of opinion such as this were infrequent. It was generally recognized that the primary purpose of a university was the advancement of learning and that an industrial concern would fail unless it was conducted to make a profit. Efforts to secure closer collaboration between the university and the industrial community in undergraduate and postgraduate work were reported from several parts of the world; it was agreed that physicists were somewhat better prepared for a practical career in countries such as Holland, where industrial scientists and engineers had a substantial role in academic research and teaching.

Discussion of the content of the curriculum embraced three main topics: (*a*) mathematics, (*b*) laboratory work, and (*c*) lectures. Commenting on the importance of adequate mathematical training, M. Y. Bernard (Conservatoire National des Arts et Métiers, Paris) proposed, not entirely in jest, that special courses should be provided for university teachers of physics, who might otherwise be embarrassed by the superior mathematical ability of their students. The consensus of opinion in France, he reported, called for three kinds of instruction. Professional mathematicians should be responsible for about 25 per cent of the undergraduate's course, dealing with topics where a rigidly logical approach was valid. A physicist should have about 40 per cent of the available time, to cover inductive methods and the development of general laws. The rest of the course should be devoted to exercise classes.

More radical views were asserted by Bernard Friedman (University of California), who condemned the "pernicious influence of sound old-fashioned courses in classical mathematical physics". Up to the end of the nineteenth century, he said, important problems giving insight into the physical world were tackled and solved by the use of special functions, separation of variables and often complicated techniques of integration. These methods were now out of date, partly because of the increasing mathematical sophistication of modern physics and partly because of the rapid advance in electronic computing. When numerical results were required, the computer would generally give more accurate results with less effort. If, on the other hand, a qualitative approach was attempted, mathematical techniques of a more abstract kind were now available.

To understand the mathematical content of present-day physics, the student must be trained in linear algebra, probability and Markoff processes, functional analysis, group representations and Lie algebras. Time does not allow the prospective physicist to follow the standard mathematical courses on these topics, but a briefer curriculum must not be allowed to degenerate into a collection of recipes for solving standard types of problem. Emphasis

should be put on the teaching of abstract concepts such as linear spaces, linear operators, convergence in metric spaces and group invariances and symmetries, even if the immediate practical applications were not fully described.

Comments on practical work were remarkable for the diversity of ideas revealed. S. C. Brown (Massachusetts Institute of Technology) claimed that the main object was to teach what a physicist does. Systematic experiments and laboratory reports should therefore be abandoned in favour of project work, closely linked with the research programme of the department. It was suggested that only the 2,000 physics students at Massachusetts Institute of Technology could ever enjoy such lavish attention, but Brown reported that most of the forward-looking universities in the United States used the same method. Some speakers thought that practical physics could be taught in lectures, though manipulative skill could only be acquired by experience at the bench. E. Mendoza (University of Wales) and F. H. Read (University of Manchester) favoured seminars in which a class (of up to fifty students) conducted one experiment under the guidance of a lecturer.

However great the importance of mathematical and practical instruction, the core of the undergraduate curriculum is in the systematic lectures by which the principles and applications of the subject are uncovered. The burdens laid on the undergraduate's knowledge and on his maturity of understanding are augmented every year. A. B. Pippard showed, from a study of examination papers, how to-day's student is expected to know just as much about electricity, thermodynamics and other long-established divisions of physics as his counterpart of 1940 —and in addition, to be conversant with many newer topics of a substantial kind. His solution was to reduce the first-degree course to two years and to provide a more comprehensive training for a proportion of the student body.

The conference passed a number of resolutions of an unexciting character, but its real significance was based on divergence rather than unanimity. Physicists are produced with evident success in educational systems sufficiently varied to include strict Government control of priorities and syllabuses in the U.S.S.R., unfettered academic enterprise in the United States and the amalgam of tradition and innovation characteristic of both the older and newer British universities. The competence and enthusiasm of the finished products encourage the belief that the confusion over aims and methods is superficial.

J. M. A. Lenihan

PLANT PHYSIOLOGY IN CANADA

THE annual scientific and business meeting of the Canadian Society of Plant Physiologists—La Société Canadienne de Physiologie Végétale—was held at the University of New Brunswick, Fredericton, during June 2–5.

The programme commenced with a symposium entitled "Problems in Marine Algal Physiology". J. S. Craigie (N.R.C., Halifax) discussed the problems of isolating and identifying marine algal excretory products from sea-water. Compounds excreted by several brown and red algae were identified as condensed polyphenolic substances with distinct growth-promoting properties. A brominated benzyl alcohol derivative excreted by *Polysiphonia lanosa* was isolated and crystallized. A pure crystalline extracellular chitin excreted by the diatom *Thalassiosira fluviatilis* was isolated and characterized and was claimed to be the first recorded evidence for the occurrence of poly-*N*-acetyl glucosamine in the algae. L. Provasoli (Haskins Laboratories, New York) discussed the nutritional requirements of marine algae grown aseptically. Unlike unicellular algae, seaweeds grown autotrophically require a range of vitamins in specific concentrations and ratios for normal morphological development. The growth of *Ulva* and *Monostroma* in aseptic culture was maintained only in the presence of plant hormones, phenolic compounds, and by the addition of supernatants containing active substances from a variety of red and brown seaweeds and from several unicellular algae grown aseptically on artificial media. These unidentified active substances are ethanol extractable and heat resistant and their ubiquitous production in the sea underlies the significance of external metabolites in the water environment. R. T. Wilce (Amherst, Massachusetts) discussed algal survival in the Arctic Sea. Reproductive activity at the end of the winter period and an abundant spring and summer growth were found to depths of 30 metres. The greatest concentration of algal biomass occurred at 15–20 metres, where extremely low light intensities were recorded. Survival under these conditions requires either the possession of a highly efficient photosynthetic apparatus or the facility to exist heterotrophically. Evidence for the heterotrophic growth of pigmented algae in deep lakes and unlit caves suggested that these arctic populations also thrived by the heterotrophic utilization of organics from the sea.

Several scientific sessions followed the symposium. Continuing their work on plant photorespiration, G. Krotkov *et al.* (Queens, Kingston) showed a distinction in spruce tissue between photo- and dark-respiration with the latter inhibited by light. W. Turner and R. G. S. Bidwell (Toronto) presented evidence for the enhancement of assimilation of carbon dioxide by leaf blades with application of IAA. E. R. Waygood *et al.* (Manitoba), working with detached wheat leaves, showed that photophosphorylation could be both restored and enhanced by benzimidazole treatment and that the synthetic pathway of NAD and NADP from ^{14}C-carbonyl-nicotinic acid was essentially the same as that reported for animal tissues and micro-organisms. The permanent bleaching of *Euglena gracilis* by nitrofuran derivatives was discussed by D. R. McCalla (McMaster, Hamilton). Other mutagens and radio-mimetic agents were used as bleaching agents, but only *N*-methyl-*N*-nitroso guanidine proved non-lethal. It was suggested that these bleaching agents damaged chloroplast DNA. Continued interest in the biosynthesis of aromatic compounds and phenolase enzyme systems was reported by A. C. Neish *et al.* from the Atlantic Regional Laboratory (N.R.C., Halifax). In particular the biosynthesis of the cinnamic acid derivatives as the C_6–C_3 intermediates in lignin formation was discussed. Other biochemical contributions of interest dealt with the *in vitro* synthesis of glycine and the metabolic utilization of ^{14}C-ethanol (E. A. Cossins *et al.*, Alberta). N. E. Good (Michigan) discussed the preparation of several new H ion buffer systems. They were mostly substituted derivatives of *tris*(hydroxymethyl)-amino methane covering the *p*H range from 6 to 8 and were claimed to be superior to both *tris* and phosphate buffers.

Several contributions described studies of the structure and the effects of environment on plant cells by electron microscope and light microscope. A. J. Mia and G. Setterfield (Carleton, Ottawa) reported the synthesis of cell wall material by apposition in *Rauwolfia* sclereids using ^{3}H-glucose pulse labelling. In a session on translocation in the higher plants, contributions from the Biosciences Division (N.R.C., Ottawa) described the experimental

control of ^{14}C-sugar movement by changing the node temperature (J. A. Webb and P. R. Gorham), and discussed the distinct lack of evidence demonstrating sugar conduction in the sieve tubes (D. C. Mortimer and M. Suzuki). D. S. Fensom and D. C. Spanner (London, England) reported their measurements of microelectrode potentials in the conducting tissue of *Nymphoides* and *Heracleum* and calculated that the electro-osmotic efficiency was sufficient to maintain sugar flow through partially blocked sieve plate pores.

In a final session on plant growth substances, several contributions were concerned with the influence of exogenously supplied gibberellins and IAA on plant development. Three reports discussed the interesting problems of IAA conjugation in plant tissues. E. Schneider and F. Wightman (Carleton, Ottawa) showed IAA–2–^{14}C to be metabolized mainly through conjugation to indoleacetyl aspartic acid, indoleacetyl glucose and 2–OH indoleacetylglucose in barley seedlings over a 24-h period. A. Winter and K. V. Thimann (Harvard University), however, could find no evidence for conjugate compounds over a 2-h period in *Avena* coleoptiles and considered fed ^{14}C-IAA to be physically bound to a protein fraction. N. A. Andreae (Department of Agriculture London) showed that conjugate products were formed with IAA inactivation, when pea roots were pretreated with an excess of IAA and NAA. An adaptive period of 2–4 h was necessary before the conjugate products were discerned. 2,4-D was not conjugated and remained inhibitory regardless of the pretreatment time. J. I. Toohey and C. D. Nelson (Queens, Kingston) reported two new herbicides derived from a soil bacterium inhabiting old pastures. The compounds were toxic to algae and higher plants but not to insects, fish or mammals. They were isolated and identified as phenazine-1-carboxylic acid (most toxic to higher plants) and the 2-OH derivative (most toxic to the algae).

Officers of the society elected for 1965–66 were: *President*, Dr. G. H. N. Towers (University of British Columbia); *Vice-president*, Dr. D. Simminovitch (Department of Agriculture, Ottawa); *Secretary-treasurer*, Dr. D. Canvin (Queens, Kingston); *Eastern director*, Dr. A. R. A. Taylor (University of New Brunswick); *Western director*, Dr. M. S. Spencer (University of Alberta). J. A. Webb

A REGISTER OF SCIENTIFIC RESEARCH IN THE NETHERLANDS

IN 1964 the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) began a register of scientific research in the Netherlands. The results of this work are to be published in the form of a periodical guide giving details of current scientific investigations. The purpose of this guide is to foster contact between research scientists. It supplies both Dutch and foreign scientists with information on the research being carried out in the Netherlands. With the view of foreign readership it appears in English.

For recent scientific information the research scientist must depend either on personal contacts or on articles in journals, reports, etc. In the case of leading scientists such as university professors working in small and highly specialized fields, personal contact often provides sufficient communication; however, this is much less true of their staff. As regards research scientists in larger and above all multi-disciplinary fields, personal contact usually satisfies only a small part of the need for communication.

So far as journals, reports, etc., are concerned, not only does it take a long time until the original publications are reported in abstracting journals, but also there is a considerable interval before the results of a research project are published. Moreover, the research scientists concerned may have information available long before then. It may also be remarked that a great deal of research is never published, for a variety of reasons, for example in the event of discontinuance of the project; valuable information may be lost in this way.

The marked expansion of research at the universities makes it still more desirable that the need for recent scientific information be satisfied. The guide contributes towards this by presenting research scientists with a basis on which further contacts can be established.

The guide has been inspired by the British publication *Scientific Research in British Universities and Colleges*, published by the Department of Scientific and Industrial Research and the British Council; this gives concise information on scientific research in Great Britain*.

At first there was some doubt about the value of this publication in Britain, but in the course of the years its utility has been clearly proved. Consequently, Z.W.O. decided to build up experience by starting in one field and submitting the result to scientists for discussion; physics was chosen as this first field.

In principle the guide only mentions research projects by or under the direct control of professors, lecturers and scientific staff.

Taking into account the purpose of the guide, preference was given to a main grouping by subject rather than by university department. As research scientists are best served by a classification that they already know, it was decided to follow the leading abstracting journal for each field of science. As the register is extended to more fields the disadvantage will have to be accepted of a certain lack of balance as compared with a general classification, such as the Universal Decimal Classification, which was devised as a whole.

For physics, *Physics Abstracts*, published by the Institution of Electrical Engineers, London, has been chosen. The research scientist was asked to classify his research project under one of the eighteen headings of *Physics Abstracts*. In order to reduce discrepancies to a minimum, a copy of the classification followed by this journal was attached to the questionnaire.

In the guide the research projects are arranged under the afore-mentioned headings of *Physics Abstracts*. Within these groups there is a sub-division according to the institution where the research is being carried out. Besides a name index and a subject index, a list of co-operating research institutes, with their addresses, is given.

Before questionnaires were sent out, the arrangement was submitted to the chairmen of the physics departments of the various universities. The idea of this was to obtain their criticism in good time, so that allowance could be made for it in the questionnaire; most of them also proved ready to call on the co-operation of the professors and lecturers to be covered by the survey.

The questionnaire was kept simple so that filling it in would not take up too much of the scientist's time. Only those questions were asked which are directly connected with the three questions which the guide tries to answer, namely: (1) What kind of research is being done? (2) Who is doing it? (3) Where is it being done?

To every professor and lecturer of physics were sent 5–10 questionnaires on which to report the relevant research; in most cases they were sent direct, though in

* Now published by the Department of Education and Science and the British Council (*Nature*, **207**, 691; 1965).

a few cases they went to a contact person for distribution to all the research scientists at a certain institution.

Although for practical reasons the first survey had to be confined to the universities, a small number of other bodies doing important physical research were also included in this first trial. Owing to the fact that investigations in borderline fields, such as physical chemistry and biophysics, are likewise done in non-physical laboratories, complete coverage was not entirely possible even within the universities; it will, however, be approached as the registration progresses.

For rapid processing of the data received—particularly with the view of future expansion to more fields—mechanization is desirable. In the case of the first guide the procedure was as follows : The copy is typed in the order in which it is received on a 'Flexowriter' which produces a punched tape as well as a typescript. After the copy has been completed the punched tape is read by a computer. This arranges the data for text and indexes in the desired order and records these on a new punched tape. The latter is then run through a 'Flexowriter' which types the manuscript for the printer without manual intervention. The pages are made up and reproduced in photo-offset.

In the preparation of the guide, Z.W.O. has received the assistance of the Statistics Department TNO for the mechanical processing of the copy and of the State Printing Department for printing. The title of the first publication, which has just appeared, is *Current Research in the Netherlands, Physics, 1965* (Pp. 66. The Hague: Netherlands Organization for the Advancement of Pure Research (Z.W.O.), 1965). The guide for physics covers about 500 current research projects on which about 750 scientists are engaged. Institutions and research scientists can obtain this publication free of charge from the Dutch Embassy in their country, or direct from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), P.O. Box 2138, The Hague.

For the following issue of the register the choice has fallen on biology; this will be registered in co-operation with the Royal Netherlands Academy of Sciences. Other sciences will follow. S. T. GROENMAN

OBSERVATIONS OF A STRONG UNIDENTIFIED MICROWAVE LINE AND OF EMISSION FROM THE OH MOLECULE

By PROF. HAROLD WEAVER, DR. DAVID R. W. WILLIAMS, DR. N. H. DIETER and W. T. LUM
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AN investigation of the galactic distribution of the OH molecule as shown by the microwave lines arising from transitions between the hyperfine levels of the ground state has led us to the following general conclusions: (1) The OH molecule is sharply confined to the galactic plane. In the plane, however, we detect OH in absorption in virtually every source brighter than a specifiable limit. (2) The OH molecule is in emission in some sources. (3) A strong unidentified emission line is present at frequency 1,665 Mc/s in a number of sources.

This discussion deals with the last two of these topics. We shall consider the galactic distribution of OH elsewhere.

Our observations were made with the 85-ft. antenna and the 100-channel switched-frequency double-comparison receiver and digital data system recently installed at the Hat Creek Radio Observatory. This system, which will be described elsewhere in detail by one of us[1], provides a spectrum consisting of 100 contiguous spectral elements which are either 10 kc/s or 2 kc/s wide. The band-width of the spectral elements is fixed by interchangeable crystal filters. The receiver includes a tunable parametric amplifier having a band-width of about 20 Mc/s. The total system noise temperature (including instrumental effects such as spill-over and the like) is approximately 130° K.

An 'observation', as the term will be used in this discussion, consists of the difference between the spectrum as observed for a specified source (integration time 1,600 sec) and the spectrum of a comparison region (integration time 1,600 sec) near the source but sufficiently far away so that the source is out of the antenna beam.

Observations of a strong unidentified microwave emission line. The Orion Nebula has been thoroughly searched for OH at 1,667 Mc/s by Robinson, Gardner, van Damme and Bolton[2]. They found no line and set an upper limit of 0·005 to the optical depth of OH in the direction of Orion. We concur with Robinson *et al.* that there is no observable line at 1,667 Mc/s. However, our observations of Ori *A* at 1,665 Mc/s show several emission components with a maximum antenna temperature of 3° K when the spectral resolution is 10 kc/s (Fig. 1).

W3, a bright extended H II region which we have also observed, shows, at 1,667 Mc/s, an absorption component of OH with a depth of approximately 1° K (Fig. 1). At 1,665 Mc/s we find, as would be predictable, the second strongest OH microwave line arising from the ground state. The depth of the 1,665 line, again as predicted, is about 0·5° K. At 1,612 Mc/s there is no absorption feature visible (Fig. 1) in the one observation we were able to make before this investigation ended. Our single observation is inadequate to show the expected OH feature at 1,612 Mc/s (predicted depth ~0·1° K), but it is adequate to demonstrate that there are no large anomalies at 1,612 Mc/s. The outstanding feature of these three spectral regions is the intense emission feature (maximum antenna temperature 8·2° K for 10-kc/s spectral resolution)

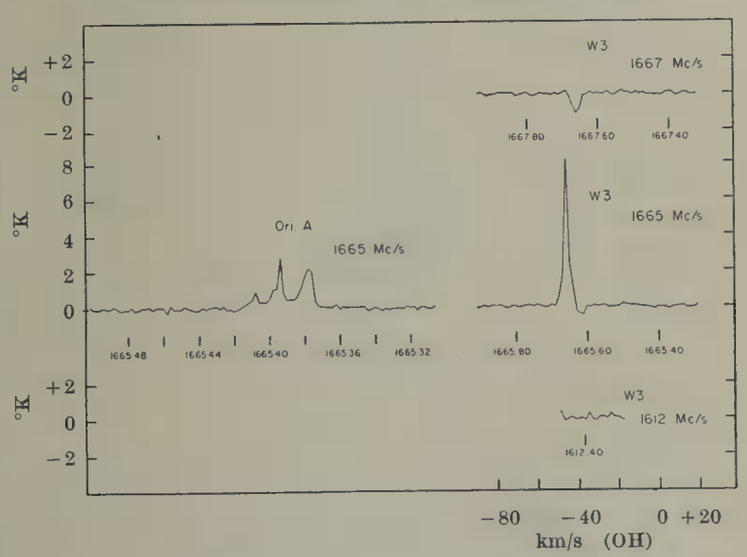

Fig. 1. Spectra of Ori *A* and *W3* with a resolution of 10 kc/s (1·8 km/sec). Frequency and velocity scales are with respect to the local standard of rest. The frequencies in the 1,667 Mc/s spectrum of *W3* should be decreased by 0·10 Mc/s. Those in Ori *A* should read 1,665·80, ·60, ·40, ·20, ·00. The ordinate is antenna temperature

upward in frequency from the 1,665 line of OH. In Fig. 2 we exhibit this strong emission line in *W3* (and the OH absorption feature downward in frequency from it) with spectral resolution 2 kc/s. With this high spectral resolution we observe a maximum antenna temperature of 31° K.

There is no known identification of the strong emission line at 1,665 Mc/s shown in Fig. 1. In what follows, for brevity in writing and to emphasize the surprising nature of the observation just presented, we shall speak of this unidentified line as arising from 'mysterium'.

We have observed 'mysterium' in two other thermal radio sources also: *W51* and *W75*. In each of these we find OH weakly in absorption and of normal intensity ratio for the lines at frequencies 1,667/1,665 Mc/s. Additionally, for each of the sources *W51* and *W75* we find a weak emission feature at 1,665 Mc/s that has no counterpart at 1,667 Mc/s. In general, in behaviour, these sources resemble the more extreme case *W3*.

Observations of OH in emission. In two sources, *W49* and *NGC* 6334, we find OH in emission. Spectra of these objects at 1,667 Mc/s and 1,665 Mc/s, spectral resolution 2 kc/s, are illustrated in Fig. 3.

W49, reported as a point source by Westerhout[3], is a heavily obscured H II region recently investigated by Akabane and Kerr[4]. On the basis of their hydrogen absorption observations they point out that *W49* is at a probable distance of 15 kpc, and that its intrinsic energy output at 1,420 Mc/s is 300 times as great as that of the Orion Nebula and one-fourth as great as that of 30 Doradus.

NGC 6334 has been less well investigated than *W49*. The *NGC* object is one of a compact group of emission nebulae which are probably optically visible parts of one object which is crossed by dense variable obscuration. Photographs of *NGC* 6334 have been published by Duncan[5]. Clark, Radhakrishnan, and Wilson[6] report a hydrogen absorption feature at +27 km/sec and infer a distance to *NGC* 6334 > 0·8 kpc. It thus appears that in the 1 Gc/s range the intrinsic energy output of *NGC* 6334 is greater than that of Orion but less than that of *W49*.

The spectra of *W49* and *NGC* 6334 are complex, consisting of numerous narrow emission maxima, some of these (as exemplified in the 1,667 line illustrated in Fig. 3) being as sharp as 5 kc/s (1 km/sec) at half-intensity. In the spectrum of *NGC* 6334 there is present OH absorption as well as OH emission.

These spectra are unusual not only because they show OH emission; they are also unusual because the intensity

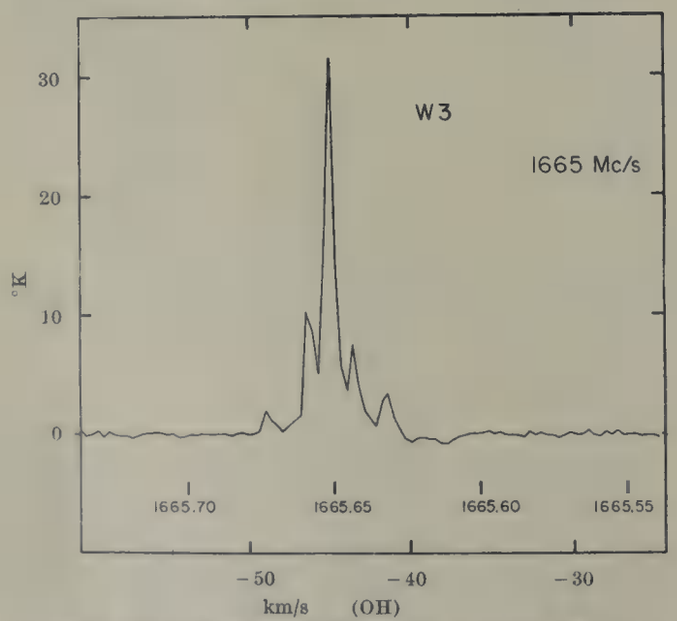

Fig. 2. Spectrum of *W3* at 1,665 Mc/s with a resolution of 2 kc/s (0·4 km/sec)

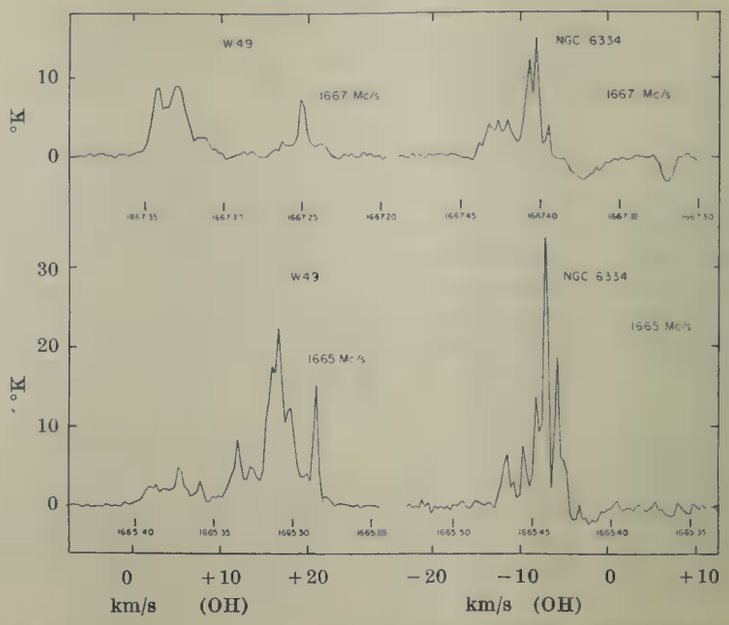

Fig. 3. Spectra of *W49* and *NGC* 6334 with a resolution of 2 kc/s (0·4 km/sec)

ratios of the emission lines appear to depart greatly from the theoretical OH intensity ratios predicted for normal thermal emission or absorption.

The theoretical intensity ratios of the OH microwave lines from the ground state, 1,667/1,665/1,612/1,720 are 9 : 5 : 1 : 1 for an optically thin gas; the ratios approach 1 : 1 : 1 : 1 as the optical thickness approaches infinity. The observed ratios of 1,667/1,665 deduced from the data in Fig. 3 are completely at variance with these theoretical values. In Fig. 3 the intensity of 1,665 exceeds the intensity of 1,667, a situation impossible in thermodynamic equilibrium.

The contour of the 1,665 line is not just the contour of the 1,667 lines scaled to great intensity. The contours of 1,665 and 1,667 are different; more numerous and more intense narrow emission maxima are present in 1,665 than in 1,667. Preliminary observations of the 1,612 Mc/s line of OH in *W49* indicate that the 1,665 contour is not reproduced in the 1,612 line.

The 'abnormal' intensities of the 1,665 lines in *W49* and *NGC* 6334 arise, we believe, from blends of the OH line at 1,665 Mc/s and the line of 'mysterium' which occurs at approximately the same frequency.

We have observed 'mysterium' where there is no OH, where there is OH in absorption, and, we believe, where there is OH in emission. We have not observed 'mysterium' in absorption.

Our observations indicate that 'mysterium' is found in strong H II regions, but not in all strong H II regions. We found no 'mysterium' in the Rosette Nebula, *M8*, *M16* or *M17*.

In the case of the Orion Nebula the 'mysterium' emission appears as a very small (possibly a point) source (the 85-ft. antenna has a beam-width of 30′) and occurs at the position of Orion *A*. In *NGC* 6334 the emission appears to come from the centre of the group of bright condensations (see the photograph taken by Duncan), not from the condensations themselves. At this centre, which is optically faint (presumably because of obscuration) we suppose the radio source to be located.

The form of the spectral lines and extreme narrowness of the emission features is striking. In the 'mysterium' emission there is at least one feature unresolved in the 2 kc/s band-width observations, and therefore not broader than 0·4 km/sec. This is the narrowest line so far observed in the interstellar medium. In a single observing period of a few hours we can see some components of 'mysterium' move from one 2 kc/s channel to another because of the doppler shift caused by the daily rotation of the Earth.

The narrowness of the features is the more surprising because the emission appears to be associated with the central regions of bright diffuse nebulae, where mass motions are typically 5 or more km/s, and temperatures are 10^4 °K. We do not know the atomic or molecular weight of 'mysterium'. We can, however, assume various weights, attribute the entire width (0·4 km/sec) to thermal broadening and compute an upper limit to the kinetic temperature of 'mysterium'. If the weight of 'mysterium' is equal to the atomic weight of He, $T_{kin} \leqslant 14°$ K; if it is equal to OH, $T_{kin} \leqslant 50°$ K; if it is equal to Ca, $T_{kin} \leqslant 140°$ K. The kinetic temperature of 'mysterium' must be very low; turbulence must be negligible.

The extraordinary sharpness of the 'mysterium' features indicates that the transition probability for the 1,665 'mysterium' transition must be quite low and that the principal energy-level involved must either be the ground state or have a long half-life.

Indications are that the rest frequency of the 'mysterium' line is higher than the 1,665·402 Mc/s line of OH. In those sources in which the two lines can be conveniently compared, the 'mysterium' line appears at a slightly higher frequency than the 1,665 Mc/s of OH in the same source.

We thank Mr. David Lesh and the staff at the Hat Creek Observatory for their assistance in making the observations and Mr. A. F. Setteducati for his help in making the reductions.

The 100-channel receiver was constructed with the aid of a grant from the U.S. National Science Foundation. The Hat Creek Observatory is operated under contract *Nonr* 222(66) with the U.S. Office of Naval Research.

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PRESSURE DISTRIBUTION IN THE TETRAHEDRAL ANVIL APPARATUS

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PRESSURES above 20 kbars cause most conventional fluids to solidify at room temperature, so that apparatus for generating pressures in this range relies mainly on solid pressure transmitting media. The use of pyrophyllite, a natural aluminium silicate, is widespread, particularly in apparatus of the 'belt' or 'anvil' types[1,2] where its high shear strength at pressure allows the formation of pressure-retaining gaskets (see, for example, Bundy, ref. 3). At the same time, its low compressibility allows pressure generation within the test cell. Since the shear strength is high, the stress system within the test cell at pressure would be expected to be neither hydrostatic nor uniform, and this is especially the case in anvil apparatus where relatively large movements of material occur during gasket formation[4]. Such variations in stress could be large, and so introduce uncertainties in pressure measurement in physical and chemical experiments at ultra-high pressure.

Stress inhomogeneities have been demonstrated in both anvil and cylindrical ultra-high-pressure apparatus, by observation of the 'smearing out' effect[5,6] produced on the normally rapid I→II resistance transition in bismuth at 25·4 kbars (ref. 7). To minimize this effect, pressure calibration specimens are usually surrounded by a 'pressure equalizing' sleeve made from a low shear strength material, typically silver chloride. Pressure within such a sleeve is expected to be reasonably hydrostatic and closely related to the mean hydrostatic stress in the surrounding pyrophyllite. By using sleeved calibration specimens at various positions within the cell (following the work of Deaton and Graf[8]), the variation of this stress has been determined for an N.B.S.-type tetrahedral apparatus[9], at pressures around the 25·4-kbar bismuth transition.

The apparatus used had steel anvils of 3·81 cm edge, to *En*26 specification, with a hardness of 530 d.p.n.; these were coated with jeweller's rouge prior to each experiment. Pyrophyllite for the tetrahedra was obtained under the trade name of 'Alsil': we have found this particular grade to be of excellent quality, machining easily, and quite free of the foreign inclusions sometimes encountered. The calibration samples in these experiments consisted of bismuth wires 1 mm diameter × 5 mm long. These were mounted in silver chloride sleeves of 3 mm outside diameter. Electrical contact was made directly to the anvil faces by suitably shaped soft copper plugs; by using all four anvils, the resistance of three samples could be followed simultaneously as the anvil load was increased.

Two sets of experiments were performed. In the first, the samples were located as in Fig. 1*a*: No. 1 was at the centroid; No. 2 with its centre on the line joining the centroid to a mid-point (half-way along an altitude) of a face; No. 3 on the normal from the centroid to an edge.

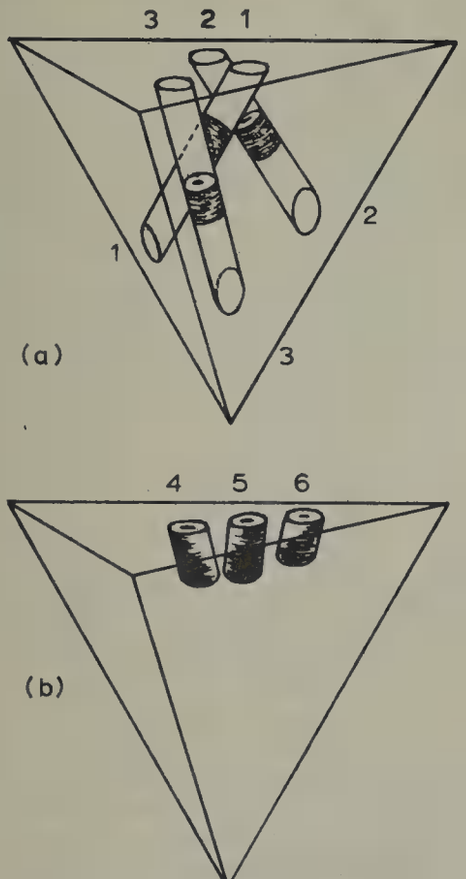

Fig. 1. Sample positions in tetrahedron. *a*, No. 1, at centroid; No. 2, between centroid and mid-point of face; No. 3, between centroid and edge. *b*, No. 4, at face centroid; No. 5, at mid-point of altitude; No. 6, towards apex (see text). The arrangement of Nos. 5 and 6 corresponds to deformed tetrahedron

Distances from the centres of samples No. 1 to No. 2 and No. 1 to No. 3 were 6 mm and 7 mm respectively. Fig. 2 shows the loads at which the 25·4 kbar transition was observed to begin at each of the sample positions, and for various degrees of tetrahedron oversize. (The variables P and V are, respectively, the transition load divided by the load necessary to produce the transition pressure over an area equal to an anvil end-face, and the precompression tetrahedron volume divided by the tetrahedron volume defined by the anvil end faces.) Maximum variation in load in repeated experiments is estimated at ± 4 per cent: I believe that this is mainly due to small differences in the mechanical properties of pyrophyllite in a given batch of tetrahedra.

It will be seen that the differing values of P in Fig. 2 imply quite appreciable pressure gradients in all cases; also that the optimum value of V (based on the minimum load to reach the transition) is much the same for all three specimen positions. Values of P and V at the minimum for sample 1 are 1·00 and 1·95, agreeing well with the corresponding results of Houck and Hutton[6] for $\frac{1}{2}$-in. and 1-in. tetrahedral units with carbide anvils.

In the second set of three experiments 4·76-cm tetrahedra ($V = 1{\cdot}95$) were used. Samples were arranged on an altitude of a face as shown in Fig. 1*b*. Sample No. 4 was at the face centroid, No. 5 half-way along the altitude from the vertex to the base, and No. 6 towards the vertex the same distance from No. 5 as the latter was from No. 4, that is ~5·5 mm. Reasonably consistent transition loads on increasing pressure were found for samples Nos. 4 and 5, typical values of P being 1·07 and 1·13 respectively. Values of P for No. 6, however, varied considerably, two samples giving 1·27 and 1·36 respectively; the third had not transformed at $P = 1{\cdot}4$ which was the maximum load applied in these experiments.

If the two sets of experiments on 4·76-cm tetrahedra are now combined, a rough picture emerges of pressure falling off fairly slowly from the centroid towards the faces, and rather more rapidly towards the edges. It is difficult to relate the values of P at each sample to corresponding pressures at each of the other samples, since we do not have a load/pressure relation for each sample position. However, an approximate relation may be derived by assuming it is of the same general shape normally observed[10], and differs only by a proportional factor in load (determined by the value of P) at each sample position. This assumption is probably not very greatly in error since the actual pressure range involved around 25·4 kbars is small. Pressures derived on this basis when sample 6 is at 25·4 kbars (the minimum observed value of P of 1·27 is used) are shown in Fig. 3 displayed with a cross-section of the deformed tetrahedron. It should be noted that the actual physical displacement of the

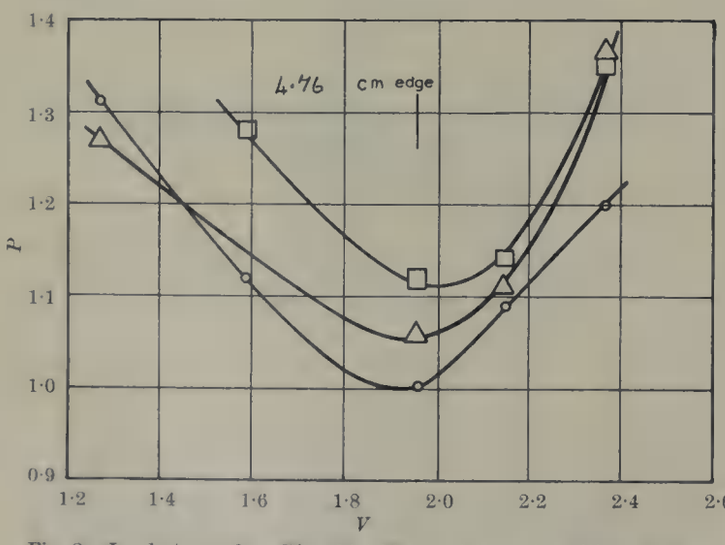

Fig. 2. Loads to produce Bi-II 25·4-kbar resistance transition at three sample positions for various tetrahedron sizes. ○, Sample No. 1; △, sample No. 2; □, sample No. 3

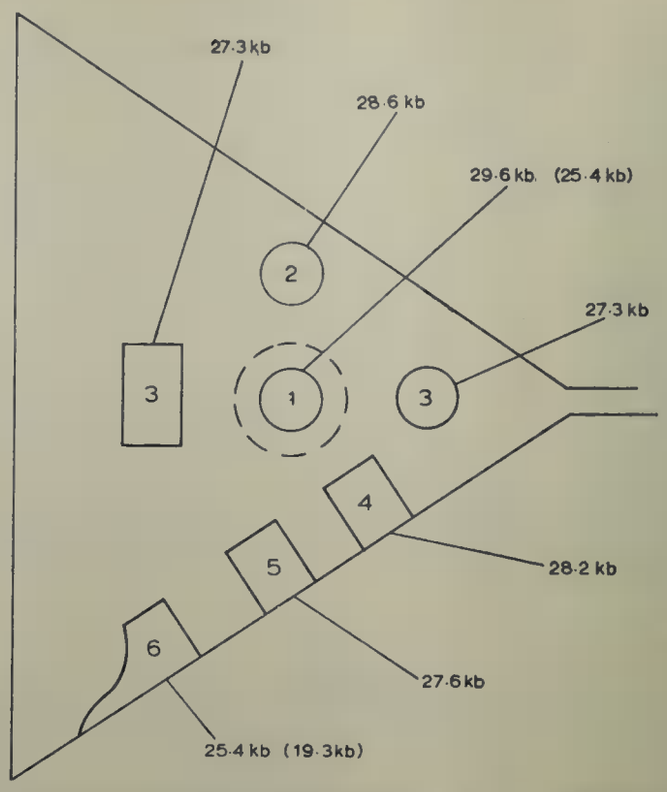

Fig. 3. Sample positions on a mid-section of the deformed tetrahedron, with corresponding pressures on loading (figures in parentheses for sample Nos. 1 and 6 are for unloading)

Fig. 4. Tetrahedron after compression, showing dark area on face

samples due to compression of the tetrahedron was found to be small, except in the case of sample No. 6 as shown. This would be expected if, as seems very reasonable, the flow pattern of pyrophyllite during compression is similar to that previously derived for a 1·90-cm edge tetrahedral apparatus[4].

The reverse transition loads on unloading were also determined, and with the exception of sample No. 1, were found to be somewhat unrepeatable. Generally, however, pressure gradients were greater during unloading. Thus, for sample No. 6, the minimum value of P for the II→I transition obtained was 1·10, compared with 0·72 for sample No. 1; the corresponding pressures are shown in Fig. 3 in parenthesis. It will be noted that, on loading, the pressure difference between No. 1 and No. 6 corre-

sponds to 14 per cent of the pressure at No. 1; on unloading the minimum difference is increased to 24 per cent.

Fig. 3 shows that in the experiments with 4·76-cm tetrahedra (*a*) there is a small but definite pressure gradient in the central regions with a larger gradient towards the edges and apices, and (*b*) gradients appear to be increased on unloading. Maximum pressure is generated at the tetrahedron centroid, thus confirming the suspected intensification effect noted by Houck and Hutton[6]; a similar effect has been observed in a cylindrical high-pressure apparatus by Tydings and Giardini[5]. Both groups of research workers ascribed the effect to possible 'cones' of undeformed pyrophyllite which act as anvil (or piston) extensions of small area, so increasing pressure in the central regions. Further evidence for 'cones' of pyrophyllite is provided by flow patterns in deformed tetrahedra[4], and by the appearance of tetrahedron after compression. The dark marks (Fig. 4) on each face are due to material which has not sheared during compression: these marks very probably represent the bases of such cones. Actual pressure differences inferred for the central regions of the tetrahedron are ~7 per cent between centroid and face. These would account for the changes previously observed in the load/pressure relation when different lengths of calibration sample are used[4].

The present results thus show gradients considerably greater in the central regions than the 1 per cent found by Deaton and Graf in their experiments at various pressures with a 1·98-cm edge tetrahedral apparatus. This could be associated with the relatively smaller pressure-sensing samples in the experiments described here (the dotted line in Fig. 3 shows Deaton and Graf's sample size on the same scale), but it could also be due to the differing absolute tetrahedron size or even to the use of steel anvils. Another possibility lies in variations in the properties of pyrophyllite: small differences which are found in the grain of machined surfaces, and in the machining properties, could well point to such an effect. Generally the pressure variations observed in the particular experiments described here indicate the need for caution in pressure calibration work, even when a pressure calibrating sample is included with the specimen under investigation: this is particularly so when the calibration sample is situated some distance away from the specimen.

I thank my colleagues K. Ashcroft and C. H. L. Goodman for discussions; also D. G. Ingham, who carried out many of the experiments.

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ELECTRIC POTENTIALS GENERATED BY FREEZING DILUTE AQUEOUS SOLUTIONS

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THE purpose of this article is to describe an investigation of the large electric potentials which occur when very dilute solutions freeze under the influence of a temperature gradient. This phenomenon is identical to the thermodielectric effect discovered by Costa Ribeiro[1]. Since the discovery of electrical potentials associated with freezing of aqueous solutions of inorganic compounds was first announced, little systematic investigation has been reported[2].

When water is frozen under the influence of a temperature gradient, if certain precautions are taken, a potential can be measured between the ice and the water phases. It was established by the work of previous investigators[2–4] that the freezing potential does not depend on the temperature of freezing alone; it depends on the rate of freezing and also on the concentration of the impurities present in the system. Most of the work published on the influence of electrolytes on freezing potentials of aqueous solutions deals with concentrations above 10^{-5} M. The few data available for solutions below that concentration show practically no electrical effects.

It was found in the course of the present work that the concentration range between 10^{-7} and 10^{-5} M presents potential values which are even larger (but of opposite sign) than those reported for concentrations above 10^{-5} M. These findings are at variance with previous works published on the influence of electrolytes[5]. The treatment of the conductivity water is probably one of the causes of this disagreement.

The phenomenon set up by the temperature gradient imposed on the system is easy to reproduce if the many parameters involved are carefully controlled. Devices used in earlier work[3] for that specific purpose probably failed to control accurately the experimental parameters on which the electrical phenomena are dependent, thus the failure to reproduce the potentials.

The results reported here were obtained with an apparatus which ensured good temperature control. The main part of the apparatus is composed of a freezing cell which consists essentially of a cup (about 1 cm³) made of a 'Teflon' sleeve that fits snugly in a brass cylinder. The top of the brass cylinder (the bottom of the freezing cell cavity) is covered with a gold plate to prevent direct contact between the brass cylinder and the solution. The cell is depicted in Fig. 1. An alumel-'Chromel' thermocouple is placed just under the gold plate, ensuring accurate temperature measurements. The cell is next placed in a cylindrical copper well (diam. 1·6 cm, depth 2 cm). The copper well is part of a thermoelectric heat pump and can be cooled down to −30° C with the loaded cell in place; the cooling unit is represented in Fig. 2. The temperature in the well (and cell) is dependent on the current flowing through the thermoelectric modulus, and the power unit supplying the modulus current is fitted with a current read out. The temperature at the bottom of the copper well is also determined with the help of a thermocouple attached to the bottom of the well; the power unit read out is then used for temperature control. The freezing potentials were measured with the help of a Keithley electrometer model (610 amp) the output of which was fed into a Varian recorder. A potential divider built with precision resistors had to be made because the potentials often exceeded the useful range of the electrometer.

A platinum wire immersed in the water phase was connected to the electrometer; the ice phase was grounded

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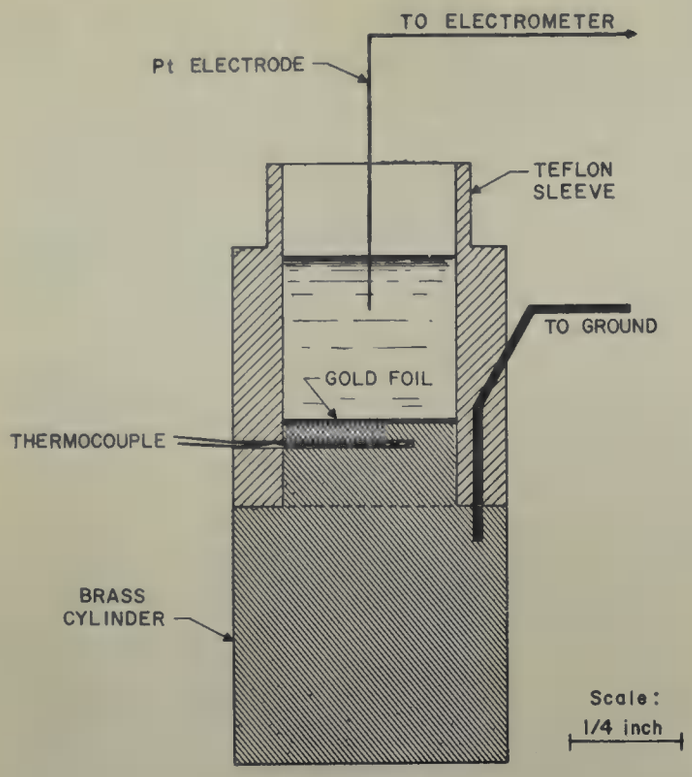

Fig. 1. Freezing cell

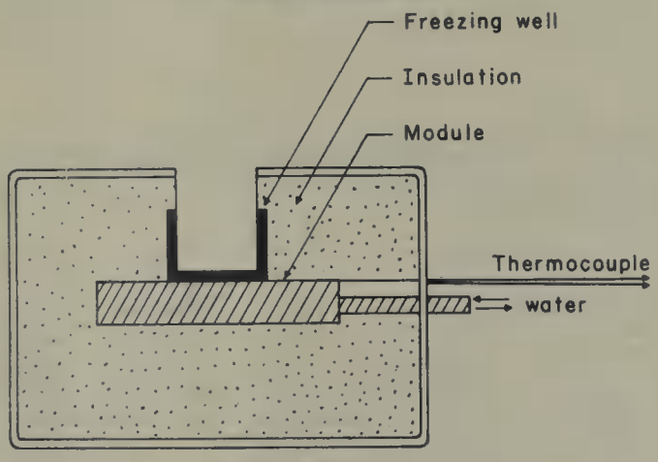

Fig. 2. Freezing well

through the bottom of the cell (Fig. 1). In this article the maximum potential values are given, that is, the highest potential value attained in the potential versus time curve. The time of occurrence of the maximum potential seems to depend on concentration, the other parameters being kept constant. For example, for 2×10^{-4} M solution of ammonium chloride the maximum potential value was observed to occur 2 min after the beginning of the experiment, whereas pure water developed the maximum value nearly 10 min after the solution was placed in the cell. The time corresponding to the maximum potential appears to increase with the decreasing concentration of ammonium chloride; no trend was observed, however, for other electrolytes such as hydrochloric acid and sodium chloride. In all cases the freezing potential value drops practically to zero at the moment of complete solidification of the water phase. In the device used in the study described here complete freezing occurs between 14 and 16 min after starting the experiment. The results reported here were obtained under the following experimental conditions: Room temperature, $24 \pm 1°$ C; brass cylinder, $-25 \pm 1°$ C; initial volume of liquid phase, 0·8 cm³; height of cell, 1·5 cm; cross-section, 0·64 cm². The solution was pipetted into the cell once the temperature of the brass cylinder reached $-25°$ C. The platinum electrode was lowered into the water phase. The potential, which appeared immediately after the solution was placed in the cup, was then measured. The details of the construction of the whole experimental apparatus were given elsewhere[6].

The effect of temperature on the freezing potentials of water and dilute aqueous solutions of electrolytes was examined earlier[3].

The effect of the addition of both organic and inorganic substances has been investigated and is briefly reported here. No effort was made to eliminate carbon dioxide, although fresh solutions were made up with recently distilled water. Bidistilled water from an all-'Pyrex' water still was used (conductivity $1{\cdot}2 \times 10^{-6}$ mho cm^{-1}). This water gave freezing potentials of $+75$ V (mean of 18 independent determinations). The freezing potential dropped to $+40$ V for once-distilled water (conductivity $1{\cdot}8 \times 10^{-6}$ mho cm^{-1}) and decreased to zero for distilled water of conductivity around 4×10^{-6} mho cm^{-1}. Generally no relationship could be found between the water conductivity and the freezing potential; however, the E_m versus conductivity plot for sodium chloride gave an excellent straight line up to 10^{-5} M (conductivity range: 1×10^{-6}–3×10^{-6} mho cm^{-1}). The E_m for dilute solutions was plotted against the logarithm for the molar concentration. Very dilute solutions of organic and inorganic substances showed a similar behaviour except solutions containing the NH_4^+ ion. The freezing potential for aqueous solutions drops to zero in the concentration range between 10^{-7} and 10^{-5} M. The potential often reverses its sign, in the case of many inorganic salt solutions, beyond $c \cong 10^{-5}$ M and drops to zero at a concentration around 10^{-3} M; however, the magnitude of the maximum potential is much smaller than that observed for the potentials measured in the dilute region (10^{-7}–10^{-5} M). The freezing potential of solutions of inorganic acids drops to zero at $c = 10^{-5}$ M and remains at zero beyond that concentration value. All organic substances investigated (sucrose, glucose, urea, etc.) also cause a drop in the potential, which is eliminated altogether at concentrations around 10^{-3} M; however, the potential sign is never reversed. The E_m versus log c plots for ammonium chloride and sodium chloride are presented in Fig. 3. Two regions can be observed in the sodium chloride curve: (*a*) a very dilute region I showing large potentials and a positive ice phase; (*b*) a region II where the ice is negatively charged with respect to the water phase but never shows the large potential differences which occur in region I. Between the two regions there is a narrow concentration range where the potential shows an erratic behaviour and very low values. The behaviour of ammonium chloride is quite different, as is shown in Fig. 3. A maximum is found at $c = 2{\cdot}5 \times 10^{-6}$ M for which $E_m = +115$ V; the potential, however, drops to zero at $c \sim 10^{-3}$ M. The chlorides of Ca^{++}, Ba^{++}, Sr^{++} and Mg^{++} also reverse the sign of the freezing potential,

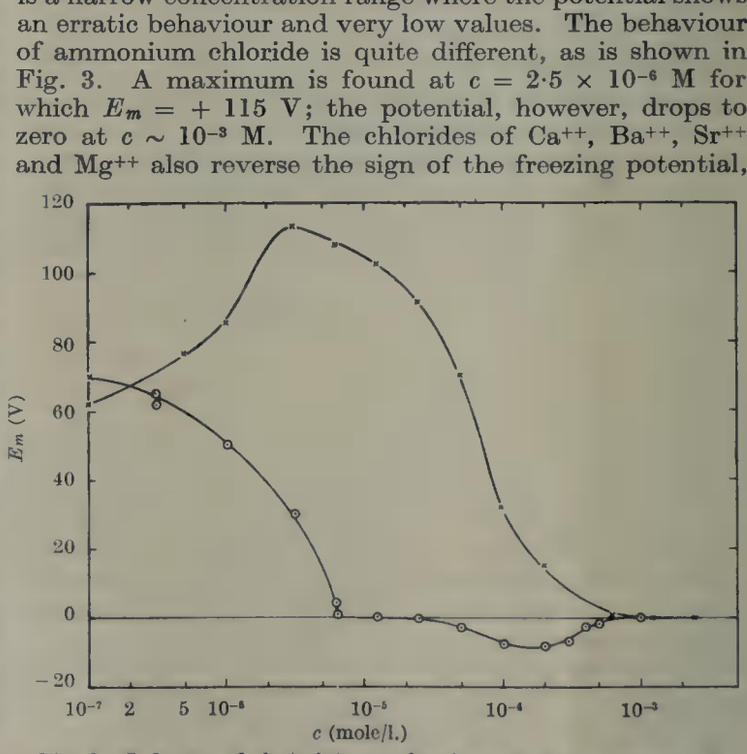

Fig. 3. Influence of electrolytes on freezing potential of water. E_m versus log c; block temperature $-25°$ C. ⊙, NaCl; ×, NH_4Cl

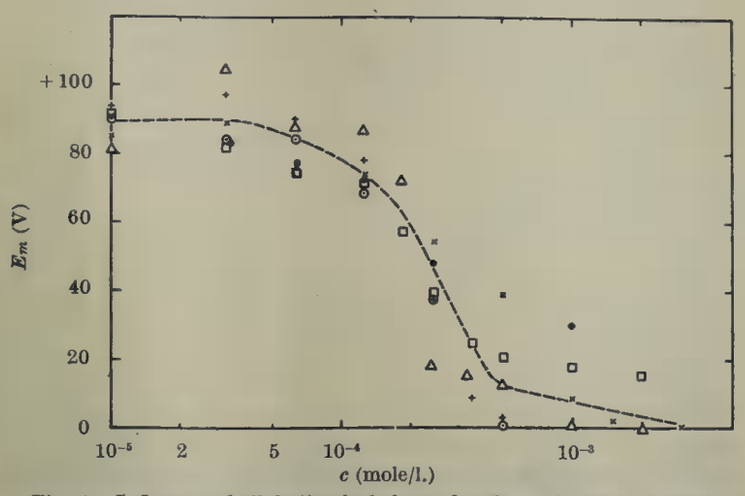

Fig. 4. Influence of aliphatic alcohols on freezing potentials of water. E_m versus log c; block temperature −25° C. ×, Methanol; ●, ethanol; ○, propanol; +, butanol; △, pentanol; □, hexanol

but do not show potential values as high as those produced by metallic uni-univalent electrolytes. The chlorides of Fe^{+++}, Al^{+++} and Cr^{+++} show only a region I; beyond 2×10^{-6} M the freezing potential is zero. The 'charge reversal' appears to be a property of uni-univalent and uni-divalent metallic salts, with the exception of those containing the NH_4^+ ion.

The large freezing potentials obtained for conductivity water are in agreement with the findings of Workman and Reynolds[3] but are at variance with the results obtained by Lodge *et al.*[5], who could not measure any potential for conductivity water passed through an ionic exchange resin column. The influence of a series of organic compounds such as sugars, glycols, alcohols, ketones and urea has also been investigated. The behaviour of aliphatic alcohols from methanol to hexanol is remarkably similar, as is shown in Fig. 4.

A theoretical interpretation of the electrical phenomena which appear during the course of a phase transition is not easy[1,8]. If all macroscopic parameters (temperature gradient, rate of freezing, orientation of the crystallographic axis of the ice formed) are kept invariant, then it is possible to ascribe the phenomenon to the following processes: (1) proton conductance; (2) dipole orientation of water molecules at the ice/water interface; (3) ionic entrapment in the ice lattice; (4) ionic diffusion.

The proton conduction process, whether taking place by quantum mechanical tunnel effect, rotation of water molecules or any other rate-determining mechanism, could be the predominant factor in the production of the freezing current, although process IV may be at play when ionic impurities are present (Fig. 3). In fact the tunnel effect has been suggested (from theoretical considerations) by others as a possible rate-determining mechanism for the proton gumping process[9,10].

Mechanism (2) may be largely responsible for the existence of the high potential values between the ice and the water phases in region I, although mechanism (1) may be operating throughout the whole concentration range investigated. Furthermore, because it is impossible to rule out completely the presence of ionic entrapment in the ice lattice, it is also possible that mechanisms (3) and (4) are also at play even at very low ionic concentrations (region I); their contribution, however, would be small in region I. The process of ionic entrapment alone does not seem to explain clearly the presence of a freezing current, as it does not explain the large potentials in conductivity water. It is possible that all processes are at play in region II, although dipole contribution would be smaller than the others. In region I the proton conduction mechanism may originate the freezing current, whereas the dipole orientation contribution at the ice/water interface may build up the large freezing potentials.

The following results were obtained for a $6{\cdot}25 \times 10^{-6}$ M ammonium chloride solution: $E_m = +110$ V (maximum potential, ice positive), $i_m = 1{\cdot}0 \times 10^{-9}$ amp (maximum current) and $q = 5{\cdot}7 \times 10^{-7}$ coulombs (1,700 e.s.u. C.G.S., total charge transferred per gram of water). The value given here for the charge is the maximum value obtained in the concentration range between 10^{-6} and 2×10^{-3} M. The values obtained by other investigators[3] (for the same substance and same concentration range) exceed by orders of magnitude the values reported here, a fact which could not be explained at the time. The use of dipole orientation to explain the appearance of the phase transition potentials is in agreement with the findings of Costa Ribeiro[1], who suggested that the ice/water interface is the site of the electrical phenomena reported here. This view was also the basis of the theoretical treatment of B. Gross[8], confirmed experimentally by Gill and Alfrey[11], Cobb[12] and by ourselves[7]. Moreover, because of chemical bond requirements between the molecules at the interface and those present in the liquid bulk phase, it is to be expected that the preferential dipole orientation of the water molecules may extend to a considerable depth. This interfacial region is thick enough to accommodate a number of layers of water dipoles. The dipole orientation occurs at both the air/water and the ice/water interface. However, the contribution of the dipoles at the air/water interface is much smaller than that of the several dipole layers at the ice/water interface because of the more random nature of the air/water interface. No surface orientation is likely to occur inside the ice phase as a consequence of the loss in configurational entropy of the ice lattice. Experiments carried out in these laboratories indicate that the dipole orientation contributions at both interfaces are being determined while the measurements of freezing potentials are made. Assuming that the surface phase (in the water) near the ice can accommodate a few layers of oriented dipoles (say, 5 layers) and that there are *c.* 10^{15} dipoles per sq. cm, a value of 22 V is calculated; an array of 15–20 dipole layers would account completely for the large potential values obtained for conductivity water. Fletcher[13], using a thermodynamic approach and elementary electrostatics, found a value of 30 V for the potential in the ice. In conclusion it seems possible that the electric potentials caused by the freezing of conductivity water and very dilute solutions are largely due to orientation of water dipoles at the ice/water interface. The predominant processes occurring in region II could be, in the decreasing order of importance: proton conduction, ionic entrapment and ionic diffusion. In region II the dipole orientation contribution would be small. The elimination of the freezing potential by organic molecules could be explained, at least qualitatively, if one accepts the presence of the proton conductance mechanism and the surface orientation of the water dipoles. The organic molecules behave as a proton trap[14], thereby eliminating the freezing current, and also as a disorganizing agent of the dipole arrays at the interface causing the complete annihilation of the freezing potential.

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RELATIONSHIP OF THE VELOCITY OF A CHARGED PARTICLE TO ITS RELATIVE BIOLOGICAL EFFECTIVENESS

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THE relative biological effectiveness (RBE) of an incident charged particle is often discussed in terms of its rate of linear energy transfer (LET). When most of the energy transferred is absorbed locally in a small target (for example, a single cell), RBE is described as a function of LET_∞, which is the stopping power $-dE/dx$ of the target for the particles[1]. Because stopping power depends only on the two properties, charge and velocity, of the incident radiation[2], RBE thus described for a particle is a function of its charge and velocity. In what follows, the 'LET dependence' of RBE will imply a dependence on both these quantities. We shall restrict the discussion to small targets in which the spatial dose distribution is uniform.

The ratio of RBE values of two radiations, RBE_1 and RBE_2, is given in terms of absorbed doses, D_1 and D_2 (energy absorbed per unit mass), by the relation:

$$\frac{RBE_1}{RBE_2} = \frac{D_2}{D_1} \tag{1}$$

In comparing the two radiations, the absorbed doses are adjusted experimentally to produce the same degree of biological effect, or end-point; other factors in the experiment, such as dose rate and species, are held constant. When a mono-energetic beam of charged particles passes normally through the face of a thin slice of tissue, the absorbed dose in the tissue can be expressed by writing $D = \varphi(-dE/dx)\Delta x/100\Delta m$. Here φ denotes the flux of particles in cm^{-2}, $-dE/dx$ is the stopping power of tissue for the radiation in ergs/cm, Δx is the thickness of the tissue slice in cm and Δm its mass in grams, and the factor 100 converts ergs/gram into rads (1 rad = 100 ergs/g). The ratio of RBE values of two kinds of charged particles, therefore, is given by:

$$\frac{RBE_1}{RBE_2} = \frac{\varphi_2}{\varphi_1} \cdot \frac{(dE/dx)_2}{(dE/dx)_1} \tag{2}$$

In recent years a number of investigations have extended and generalized dosimetric concepts to cover a wider range of phenomena than before[3]. One group of investigations has led to a quantitative description of RBE in terms of the physical properties of radiation for certain biological end-points[4]. This theory, which appears to describe the RBE of neutrons, photons, electrons and singly charged ions, and to give reasonable results for α-particles, implies that RBE depends only on the velocity of a charged particle rather than LET[5]. The theory thus implies that the RBE of a charged particle is independent of the particle's charge. This finding differs from the conventional description of RBE[1].

The present article attempts to clarify the question of charge dependence of RBE by the application of general principles of collision theory. Although the interactions and mechanisms that lead from an initial physical event between radiation and matter to biological damage are doubtlessly complex, the way in which charge enters the picture is given explicitly in the formalism of quantum mechanics. (The same is also true, of course, in classical mechanics.) In particular, the formalism of successive Born approximations gives an expansion of exact scattering amplitudes in powers of charge. This permits making a definite choice between velocity dependence and LET dependence on theoretical grounds within well-defined limits known from quantum mechanics. As the following analysis shows, the validity of the limits of charge independence of RBE appears to be identical to the limits of the validity of the first Born approximation in collision theory.

Non-relativistically, an incident particle having a charge $+ze$ interacts with an atom consisting of electrons of charge $-e$ and a nucleus of charge $+Ze$ by means of the Coulomb energy operator:

$$V = -\frac{zZe^2}{|\vec{r} - \vec{R}|} + \sum_{j=1}^{Z} \frac{ze^2}{|\vec{r} - \vec{r_j}|} \tag{3}$$

Here $\vec{r}$ denotes the position vector of the incident particle, $\vec{R}$ that of the nucleus, and $\vec{r_j}$ that of the j^{th} electron. The sum is taken over all electrons ($j = 1, \ldots Z$) in the atom. The transition probability, w_{no}, for excitation of the atom from one state o to another state n with the simultaneous transfer of momentum (and energy) from the incident particle is, in the first Born approximation, proportional to the square of the matrix element of V taken between the initial- and final-state wave functions, ψ_o and ψ_n, of the system, atom-plus-particle. The operator $1/|\vec{r} - \vec{R}|$ has zero matrix element between different (orthogonal) states depending on $\vec{r_j}$, and so only the second term in equation (3) contributes to w_{no}. The transition probability is proportional to the square of the charge of incident particle:

$$w_{no} \sim |<\psi_n|V|\psi_o>|^2 \sim z^2e^4|<\psi_n| \sum_j \frac{1}{|\vec{r} - \vec{r_j}|} |\psi_o>|^2 \tag{4}$$

where the symbol $<||>$ denotes the matrix element for the operators and wave functions shown. The total cross-section σ_i for the interaction of a single incident particle per cm^2 with one atom of type i per cm^3 is calculated by integrating w_{no} over all possible scattering directions and then taking the proper average over initial states and sum over final states of the system. The charge dependence shown in equation (4) is not affected by these operations and so we may write:

$$\sigma_i = z^2e^4 f(v) \tag{5}$$

where $f(v)$ is a function depending on the velocity, but not the charge, of the incident particle.

The stopping power of the medium is closely related to σ_i. It is calculated by first multiplying w_{no} by the excitation energy of the transition from state o to state n and then carrying out the foregoing operations, after which the result is weighted by the number of atoms N_i of type i per cm^3 in the medium and summed over i. Explicit evaluation of the stopping power[2] gives an equation of the form:

$$-\frac{dE}{dx} = z^2e^4 g(v) \tag{6}$$

where $g(v)$ is, for a given stopping medium, a universal function of velocity v for all charged particles. (The function $g(v)$ is given explicitly, for example, by equation (38) of ref. 2.) The expressions (5) and (6) also hold relativistically in the first Born approximation. The inclusion of the second and higher approximations destroys the homogeneity of these expressions in the incident particle's charge ze.

In order to relate the discussion to RBE, a connexion has to be made between the occurrence of a physical collision on a microscopic scale and the occurrence of observable biological damage. We assume that the amount of damage done to a biological system is related to the number of primary quantum transitions or 'hits' which occur between the incident radiation and the target. In keeping with the definition of RBE, we shall assume only that the same extent of damage is produced by two charged radiations (other conditions being the same) when the number of primary quantum transitions per unit volume in the systems is the same. (Since we are considering small targets with uniform dose distribution, this approach is equivalent to relating biological damage to the total number of primary transitions in the system. The volume of the system would then appear explicitly in this description, but would cancel later from both sides of equation (7).) This assumption does not imply a quantitative relationship between degree of damage and number of transitions, but states merely the biological equivalence of two radiations producing the same end-point. This criterion for damage need hold only statistically, because the number of primary quantum transitions leading to observable damage is large.

Since the number of primary transitions per unit volume in a system due to a flux φ is $\varphi\Sigma_i N_i\sigma_i$, we describe equal biological damage from two radiations by writing:

$$\varphi_1 \sum_i N_i\sigma_{1i} = \varphi_2 \sum_i N_i\sigma_{2i} \qquad (7)$$

where σ_{1i} and σ_{2i} are the values of σ_i for the two radiations. The resulting flux ratio, when substituted into equation (2) for RBE, gives:

$$\frac{\mathrm{RBE}_1}{\mathrm{RBE}_2} = \frac{\sum_i N_i\sigma_{1i}}{\sum_i N_i\sigma_{2i}} \cdot \frac{(\mathrm{d}E/\mathrm{d}x)_2}{(\mathrm{d}E/\mathrm{d}x)_1} \qquad (8)$$

The charge dependence of this ratio can be written at once from equations (5) and (6):

$$\frac{\mathrm{RBE}_1}{\mathrm{RBE}_2} = \frac{z_1^2 e^4 \sum_i N_i f(v_1)}{z_2^2 e^4 \sum_i N_i f(v_2)} \cdot \frac{z_2^2 e^4 g(v_2)}{z_1^2 e^4 g(v_1)} = F(v_1, v_2) \qquad (9)$$

The explicit dependence on the charges of the incident particles cancels, and it follows that the ratio of RBE values can be expressed as a function $F(v_1,v_2)$ depending only on the velocities of the particles. This is the principal result of the work described here.

While it is true that a highly charged ion causes more damage (that is, a larger number of primary quantum transitions) than a singly charged one at the same velocity (lower LET), it does not follow that more damage results per unit absorbed dose. Whereas the stopping power of an ion is proportional to z^2, the flux needed to produce a given number of transitions is proportional to $1/z^2$. The magnitude of the charge acts as a coupling constant for the strength or intensity of the interaction between radiation and matter. Its effect cancels when different radiations are compared on the basis of their absorbed doses, as equation (2) for RBE specifies.

The foregoing results also hold relativistically in the first Born approximation, which coincides with the limits of accuracy of Bethe's original stopping power theory[6] including various corrections[2]. Physically this means that the theory is valid so long as the speed of the incident particle is much greater than the speed of atomic electrons. For light elements, which are the chief constituent of soft tissue, the first Born approximation, particularly when it includes shell corrections, is known experimentally to give an adequate description of charged particle slowing down for radiation of practical interest in radiobiology. For protons in water, for example, the theory is adequate down to energies of less than 1 MeV (ref. 7). Direct experimental evidence for the possible failure of the first Born approximation appears to have been found only recently, in connexion with the $\Sigma\pm$ hyperon mass anomaly[8]. The second Born approximation introduces a difference in the stopping powers of positive and negative particles that are otherwise identical. The observed difference is of the order of a few per cent.

In summary, it appears that the validity of charge independence for RBE is equivalent to the validity of the first Born approximation in quantum mechanics. Within the framework of this approximation, the concept of 'LET' dependence for RBE should be replaced by the more precise 'velocity' dependence. Higher-order approximations are needed to describe particles at lower speeds[9], and charge cannot be factored out and cancelled from the resulting expressions. Experiment indicates, however, that corrections resulting from inclusion of higher-order approximations may amount to only a few per cent.

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ADENOSINE TRIPHOSPHATE–METAL–NOREPINEPHRINE TERNARY COMPLEXES AND CATECHOLAMINE BINDING

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CATECHOLS and ethanolamines have been shown to form relatively stable chelate bonds[1–3], and metals have frequently been associated with catecholamine activity[4–8] so that a reasonable approach to the study of catecholamine binding would seem to be from the point of view of co-ordination chemistry. With the catecholamines a wide variety of chelate species are possible and the different structures would have varied

electrostatic charges and solubilities. Some of these species have recently been elucidated by Walaas *et al.*[9] in work on Cu(II) chelates of norepinephrine (NE) and epinephrine in connexion with ceruloplasmin. In anaerobic systems the Cu(II) NE chelate was found stable whereas under aerobic conditions oxidation occurred at a moderate rate, which was in agreement with the findings of Chaix *et al.*[10].

In this article results are presented which suggest that co-ordination complexes may be of major importance in the understanding of the biological phenomenon of binding of the catecholamines. This possibility was independently derived by Carlsson *et al.*[6], but set aside when by paper chromatographic methods sufficient quantities of metals were not found in adrenal medullary particles.

In the work recorded here the levels of metals were determined in sub-cellular fractions of brain known to have high concentrations of the catecholamines. Adult male mice were killed by decapitation, the brains quickly removed, the cerebellum and cerebral mantle dissected away and the remaining brain tissue (the medulla, pons, mesencephalon, diencephalon, hippocampii, and pyriform cortices) was homogenized in 0·32 M sucrose; the technique of Gray and Whittaker[11] was used to obtain synaptosomes, and the method of DeRobertis *et al.*[12] was used to obtain synaptic vesicles. In the sub-cellular fractionation the method was slightly modified in that the sucrose (Baker and Adamson reagent grade) was washed with glass-distilled water on a Buchner funnel, dried, dissolved with water to 1·2 molar, and passed over a washed 'Dowex 50' column in the hydrogen form. Succinic acid dehydrogenase (SDH)[13] and NE levels[14] were determined for the myelin, synaptosome, and mitochondrial fractions to determine the degree of separation of the mitochondria from the synaptosomes. The metals quantitatively measured were magnesium, iron, copper and zinc. For the metal analyses the sub-cellular fractions were dried under vacuum, ashed at 600° C, dissolved in N hydrochloric acid, and measured against standards in an atomic absorption spectrometer (Perkin-Elmer model 303). From the results of these analyses, shown in Fig. 1, it may be seen that in general a good share of the metals in brain are present in the nerve endings. Moreover, while the metals present in this fraction tend to be in proportions similar to the proportions found in brain, copper seems to be concentrated several-fold relative to the other metals. These findings indicate that the metals magnesium, copper, iron and zinc are present in synaptosomes in sufficient quantities for chelation to be of biological significance.

Metal levels for the synaptic vesicles were determined, in addition, as described above. In this case the metals were again found present in good yield and the level of copper was again noted to be increased relative to the amounts of other metals (Table 1). These findings suggest that metals are present in structures associated with NE rather than with unrelated proteins in the synaptosomes.

Table 1. Metal Values for Synaptic Vesicles are expressed as μg per Synaptic Vesicles obtained from 1 g Whole Brain ± *S.E.M.*

Metal	Whole mouse brain (μg/g)	Synaptic vesicles (μg/g)
Mg	116 ± 1·7	14·05 ± 0·29
Fe	51·7 ± 3·9	1·7 ± 0·56
Zn	18·7 ± 0·8	1·05 ± 0·05
Cu	10·7 ± 0·5	2·0 ± 0·16

In summary, both quantities and intracellular location of metals support the hypothesis that co-ordination between metal and NE in the synapse is of biological significance. It therefore seemed of interest to test the effects of a chelating agent on the uptake of exogenous NE. The test materials used were synaptic vesicles rather than synaptosomes and the chelating agent used was ethylenediamine hydrochloride. The control system contained ethylamine hydrochloride at twice equimolar concentration to standardize for ionic effects[4,15] and possible reactions between amines and quinones even to a limited degree[16]. The results obtained in this experiment are shown in Table 2 and the data clearly indicate that the uptake of the exogenous NE was inhibited by this chelating agent. These findings represent additional support, therefore, for chelation as a mechanism for the binding of catecholamines.

Table 2

	No addition	0·6 mμM *d,l*-NE	0·6 mμM *d,l*-NE plus 3·2 μM ethylamine hydrochloride	0·6 mμM *d,l*-NE plus 1·6 μM ethylenediamine hydrochloride
mμg NE in synaptic vesicles after incubation at 0° C for 30 min	14·4*	39·6 ± 1·4	34·0 ± 1·1	17·6 ± 4·0

Synaptic vesicles were prepared from 4 g whole mouse brain, divided into 4 equal aliquots, and brought to 1 ml. with 0·32 M sucrose + additions as noted. No. = 5 each value. Values are expressed as mμg NE ± *S.E.M.*

* To obtain this value the NE released from synaptic vesicles during the incubation (3·4 ± 0·2 mμg) was subtracted from the endogenous level without incubation (17·8 ± 0·8 mμg).

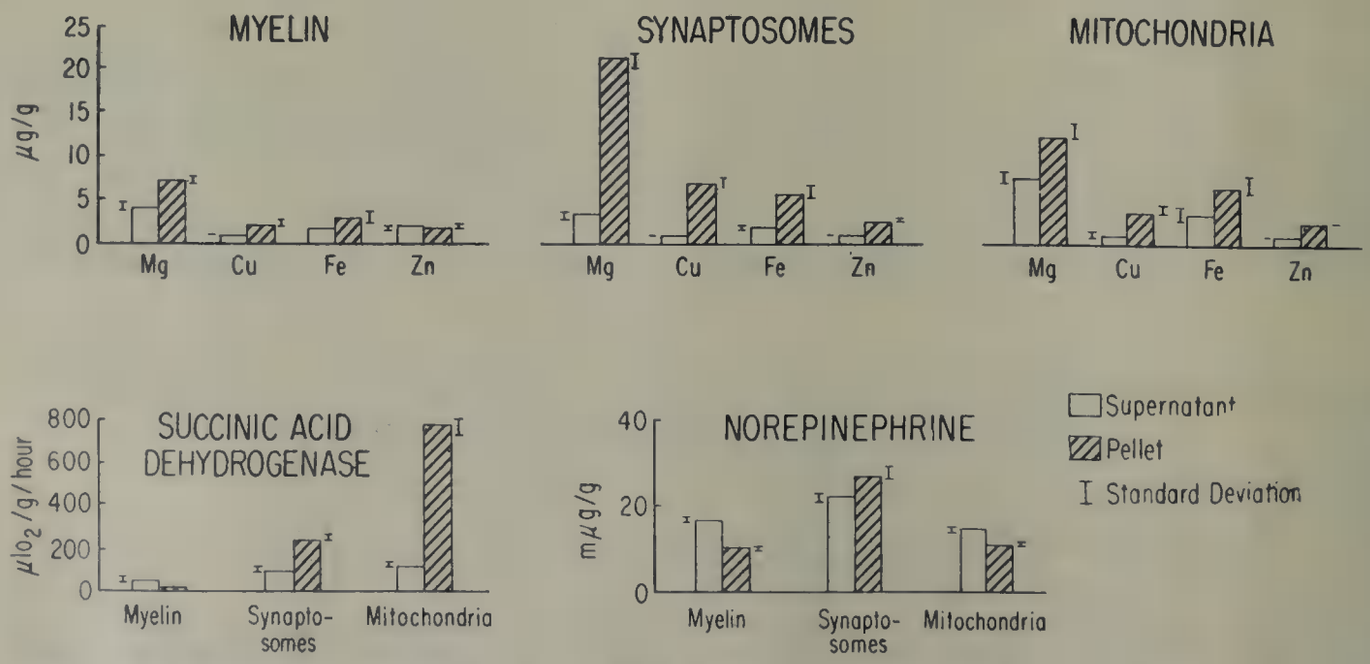


Fig. 1. Metal, SDH and NE levels in sub-cellular fractions of dissected mouse brain. NE was determined by a modification of the method of Anton and Sayre (ref. 14). Metal values are expressed as μg sub-cellular fraction obtained from 1 g of original tissue. SDH and NE values as μl. O_2/sub-cellular fraction from 1 g original tissue/h and mμg/sub-cellular fraction from 1 g original tissue respectively

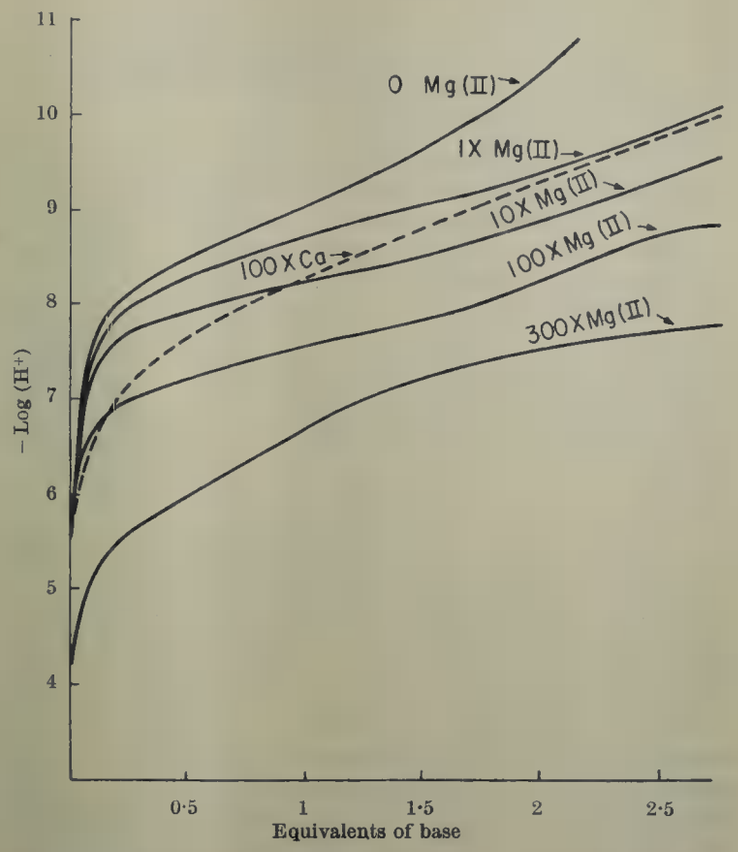


Fig. 2. Potentiometric titration of NE (0·1 mmoles in 10 ml. aqueous solution of 0·1 M KCl, 0·02 M NaCl) with increasing molar ratios of Mg(II) and Ca(II) with carbonate-free 0·1 N KOH

To study the co-ordination chemistry of the metals, magnesium, copper and iron with NE, the method used was potentiometry. This technique was thought best for this survey since it is generally applicable with this class of compounds and since the degree of chelation may be estimated directly from the titration curves. When NE was titrated with and without magnesium in increasing molar ratios it was found that with equimolar magnesium the chelation was negligible at physiological *p*H, but that with increased molar ratios of magnesium the chelation increased to levels easily of biological significance (Fig. 2). The titration curves for NE with Ca(II) at a molar ratio of 100, shown in addition in Fig. 2, indicate that NE forms somewhat less stable chelates with Ca(II) than with Mg(II). It would be of interest to determine stability constants for NE from the above titration curves, but since chelation may occur at either the catechol moiety or ethanolamine side-chains, additional data are required for such calculations.

For the titration of NE with Cu(II) and Fe(II) it was found necessary to use an organic solvent since the chelates formed were insoluble in water over the *p*H range examined. The solvent found suitable was water : propanol : butanol 2 : 3 : 2, and the electrodes were standardized with *tris* buffer in this solvent. The titration curves obtained in this case with NE and Cu(II) and Fe(II) indicate that at physiological *p*H, NE essentially completely chelates the metal present even at equimolar metal concentrations (Fig. 3).

In addition it seemed of interest to titrate ATP plus metals with and without NE present, since ATP has recently been found concentrated in synaptosomes[17] and since ATP has been associated with NE storage in many studies[4,6]. A reasonable hypothesis in this case seemed to be that ATP, metal, and NE might form an ATP-metal-NE ternary complex and that such complexes might be of particular importance in the reaction mechanisms of catecholamines. During the titration of the samples containing Cu(II) and Fe(II) with NE present, it was found that additional protons were released through the *p*H range 6–8 (Fig. 4). Since NE normally releases protons only at *p*H values > 7·5, this, and the formation of an intense blue colour in the case of Fe(II), indicated that a good proportion of the NE was chelated at *p*H 7·4. The question remaining was whether NE displaced the ATP or chelated with additional co-ordination positions of the metal. It was therefore of interest to note that no precipitate was observed in the samples containing either iron or copper with NE and ATP, whereas a precipitate forms when NE alone is in solution with copper or iron (see foregoing). This finding, in conjunction

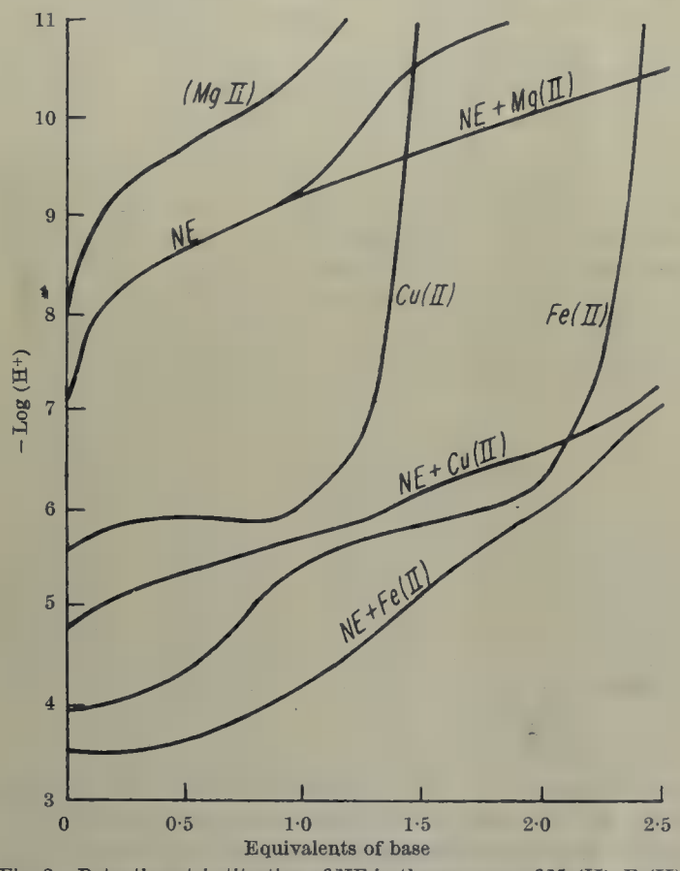


Fig. 3. Potentiometric titration of NE in the presence of Mg(II), Fe(II) and Cu(II) (equimolar, 0·05 mmoles in 100 ml. of solvent) with carbonate-free 0·1 N KOH. Solvent system, water : propanol : butanol, 2 : 3 : 2

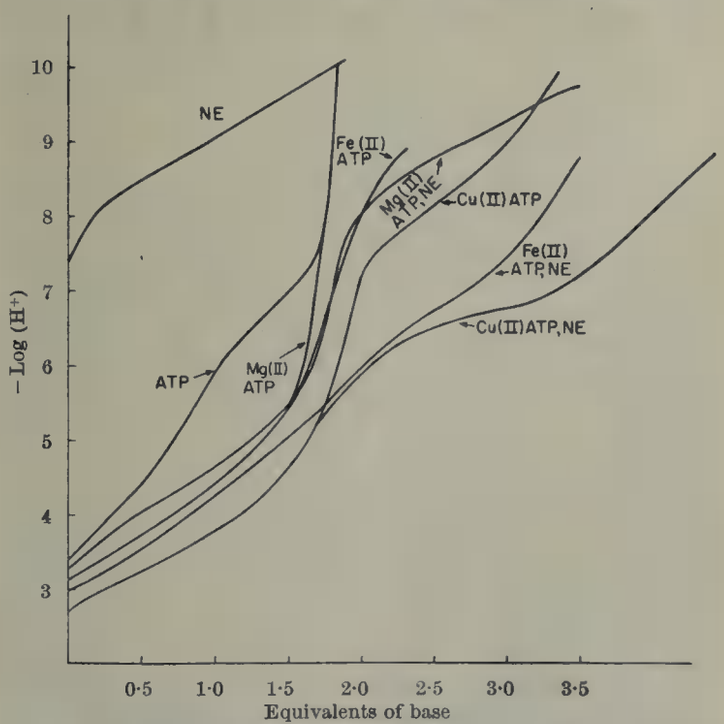
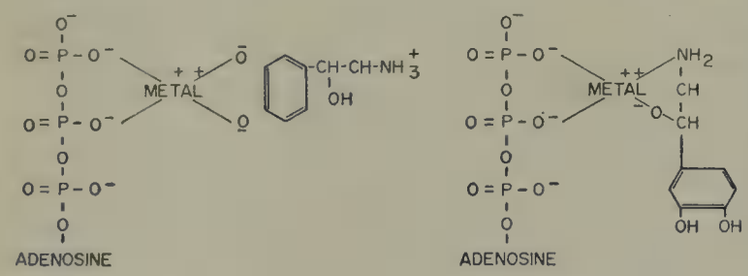


Fig. 4. Potentiometric titration of NE in the presence of various combinations of ATP, Mg(II), Cu(II) and Fe(II) (equimolar, 0·1 mmoles in 10 ml. aqueous solution of KCl, 0·02 M NaCl) with carbonate-free 0·1 N KOH

with the colour formation and the titration curves, is good evidence for ternary complex formation.

If ternary complexes of the type ATP-metal-NE structures form *in vivo*, a reasonable hypothesis would seem to be that such complexes represent a mechanism for storage or binding of catecholamine molecules. This general hypothesis will be considered further in the remainder of this article in terms of possible structures for the complexes and explanations of a variety of reported findings regarding catecholamine storage.

In recent studies of metal ATP complexes, Cohn and Hughes[18] presented evidence that copper co-ordinates with ATP at either the α- or β-phosphate groups and to some, as yet uncertain, degree with the adenine ring. Magnesium was found to chelate with the α- and β-phosphate groups while zinc apparently chelates with the adenine ring. This, therefore, allows for an even greater variety of possible chelate species which may be significant in catecholamine binding. Structures representing typical ternary complexes might involve either the catechol moiety (I) or the ethanolamine side-chain (II). In the structures shown, negative and positive charges are indicated, but with co-ordination and hence covalent bond formation, the charge distributions of the complex will be altered and at some co-ordination positions the positive and negative charges would be essentially cancelled.



I. II

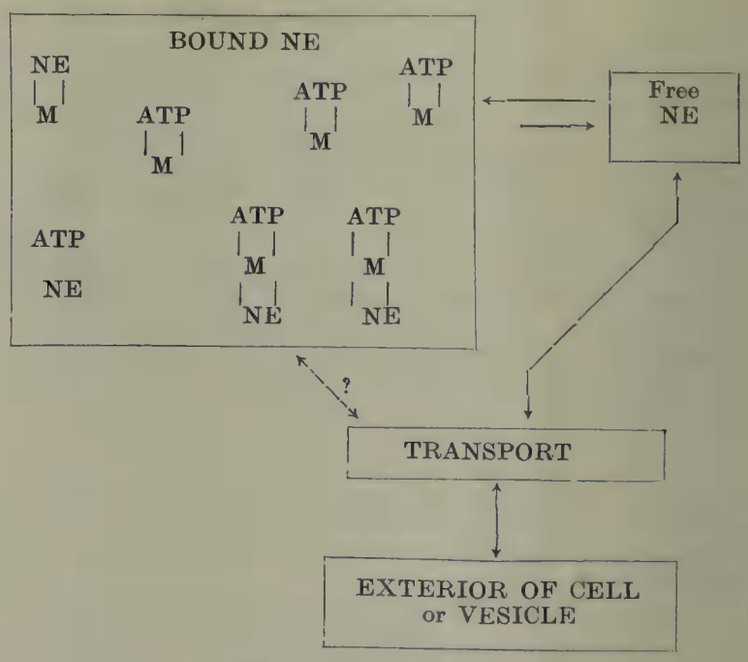
The structure of these complexes should be considered briefly in terms of possible oxidation of the NE in the presence of the metals[10]. Since the oxidation of catechols in the case of copper depends on the formation of the percupryl complex Cu OO–, which in turn depends on hybridization of the copper orbitals to form a tetrahedral structure[19], a reasonable hypothesis seems to be that in the ternary complex the ATP either competitively blocks the co-ordination of oxygen or that it does not promote formation of the tetrahedral structures. In our experience so far, norepinephrine at physiological *p*H in the ATP–Fe–NE complex in 0·1 molar potassium chloride and 0·02 molar sodium chloride does not oxidize for several hours.

Several reported experimental findings dealing with the storage of the catecholamines may be explained in terms of binding through complexes of the ATP–metal–catecholamine type. During investigations with rat heart, for example, Musacchio, Kopin and Weisse found that the binding strength of amines was in the order NE > dopamine > octopamine > tyramine[20]. That is, with increased hydroxylation there is increased binding. Moreover, *O*-methylated compounds were much weaker binding agents than the analogous hydroxy amines. Similar findings have been made with rat brain[21]. This order of binding follows directly the order of the stability constants which may be estimated in general for such compounds[3].

Tyramine displacement of NE[22] at first seemed contradictory to a chelation hypothesis for binding, but Musacchio, Kopin and Weisse[20] have shown that tyramine is metabolized to octopamine which has the ethanolamine side-chain before it becomes an effective competitor for the storage sites.

A further interesting finding which is consistent with the foregoing hypothesis has been reported by Von Euler *et al.*[23]. The uptake of exogenous NE by splenic nerve granules was found to increase essentially linearly between *p*H levels 6 and 8·5. This apparently is the precise *p*H range already shown here (Fig. 4) over which NE chelates with Fe(II)-ATP and Cu(II)-ATP.

Tissues rich in NE have the capacity to take up this amine from exogenous sources against a concentration gradient[6,24]. This finding may be explained in terms of a chelation hypothesis as follows. Calculating from data in Table 1 using only zinc, copper and iron (excluding magnesium), one may estimate that the synaptic vesicles from a gram of brain have enough metal to co-ordinate with approximately 0·08 μM of NE (assuming one NE per metal and essentially complete chelation). Even though this calculation is a rough one, it does indicate that metals are present far in excess of the NE. As such, many storage sites (co-ordination positions of metal occupied by water or other molecules which can be displaced by NE) are available for chelation with NE. Owing to the loss of NE from vesicles during preparation, even more positions would become available for NE. As a consequence of this, NE which enters a vesicle would be expected to complex with metal or metal-ATP. The excess of metal compared to NE, by the law of mass action, would favour the development of the chelate species which, assuming the chelate remains intravesicular, would produce a concentration against a gradient. In this formulation the concentrations of metal, NE, and the stability constants will determine the point at which apparent 'saturation' of binding sites appears. Schematically the binding of NE may be represented as follows (M = metal):



A role for magnesium and ATP in the uptake of NE[4,6] has so far been ignored but will be considered in the following article. However, it should be noted here that Kirshner[4] found that in the presence of zinc + ATP and aluminium + ATP the uptake of isotopically labelled NE was inhibited. This may be explained in terms of a chelation hypothesis, as these metals would be expected to form ternary complexes (ATP–M–NE) which would compete for free NE, hence reducing the amount of uptake by the granules.

In summary, evidence has been presented indicating that metals, copper in particular, are concentrated in nerve endings and synaptic vesicles in quantities easily sufficient to form chelates with catecholamines. In addition, the chelating agent ethylenediamine hydrochloride was shown to effectively block the uptake of exogenous norepinephrine. Potentiometric evidence has been given which indicates that norepinephrine chelates with metal–ATP complexes to form a ternary metal–

ATP–NE complex. A unifying concept was then constructed with metal or metal–ATP complexes representing the intravesicular binding sites for catecholamines. The way in which such a hypothesis might explain a variety of reported experimental findings was outlined.

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CO-ORDINATION CHEMISTRY AND MEMBRANE FUNCTION WITH PARTICULAR REFERENCE TO THE SYNAPSE AND CATECHOLAMINE TRANSPORT

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IN an earlier investigation[1] results were presented which indicated that a fraction of pinched-off nerve ending particles (synaptosomes) and synaptic vesicles from brain tissue contained appreciable quantities of copper, iron, and magnesium. The significance of this finding, viewed from the perspective of co-ordination chemistry, for the mechanism of binding of norepinephrine (NE) was discussed. It was also noted that these metals were not freely soluble but tended to be associated with the particulate fraction. This finding suggested that these metals might be co-ordinating with membrane constituents and hence be importantly related to membrane structure and function. Data and theory bearing on this possibility are presented and discussed in this article. In addition, the manner in which such co-ordination structures might be related to the binding and transport of NE is further explored.

Phosphatidyl-1-serine (PS) was obtained from Nutritional Biochemical Corporation, phosphatidyl inositide (PI or fraction I of Folch) and adenosine triphosphate (ATP) as the disodium salt from Sigma Chemical Co. The phospholipids used are constituents of cell membranes in general and have been reported to be present in the membranes of synaptosomes and synaptic vesicles[2]. Sub-cellular fractionation of brain tissue was done as follows: Male rats of approximately 90 days of age were killed, the brains quickly removed, the cerebellum and cerebral mantle dissected away and the remaining brain tissue homogenized in 0·32 M sucrose cleaned of metals as previously noted[1]. The technique of Whittaker[3,4] was used to prepare a fraction of synaptosomes. Evidence that this fraction was composed of synaptosomes was obtained by a spot check of succinic acid dehydrogenase activity (SDH)[5] and NE content[6] of the myelin, synaptosome, and mitochondrial fractions. The SDH activity in $\mu 10_2$/g original tissue/h was 18, 60 and 793 respectively. NE values in mμg/g original tissue were found to be myelin: 7·5; synaptosomes, 52; and mitochondria, 41·5. It is assumed that the NE levels for the mitochondrial fraction represent a contamination by synaptosomes. Lipid extracts of pellets of synaptosomes were prepared by the method of Folch, Lees, and Sloane-Stanley[7]. An upper phase non-lipid fraction was also obtained by this method. This fraction is hereafter referred to as 'upper phase extract of synaptosomes'. The fractions were evaporated to dryness and resuspended in water. Titrations and metal determinations were carried out as previously noted[1]. The solubility characteristics of various metal complexes in aqueous-ether phospholipid dispersions were obtained as follows: The combination of compounds to be examined were added in noted amounts to glass-distilled water and then the particular metal being used was added and the total volume brought to 1 ml. The *p*H was then adjusted to 7·3 with sodium hydroxide. This aqueous phase was then overlain with 3 c.c. of diethyl ether, the tubes capped, shaken for 15 min, and centrifuged at 2,500 r.p.m. for 15 min. At this point the colour of the ether phase was noted and 0·4 c.c. of the ether phase was removed for metal assay. If NE values were to be determined, tritiated *d,l*-NE (New England Nuclear Corp.) was mixed in tracer amounts with cold *d,l*-NE in noted quantities. C.p.m. were determined from both aqueous and ether phases using a Packard liquid scintillation spectrometer, and partition coefficients calculated as:

$$\frac{\text{c.p.m./c.c. ether phase}}{\text{c.p.m./c.c. aqueous phase}}$$

The results obtained may be summarized as follows. When phosphatidyl-1-serine is titrated in the presence of Mg(II), Cu(II) or Fe(III) the curves obtained indicate co-ordination of these metals with the phospholipid (Fig. 1). Moreover, evidence for complex formations between metal and phosphatidyl-1-serine altering the physical characteristics of the components is indicated in Table 1, in which it is noted that the metal content of the ether phase increases. If ATP is added to the phosphatidyl-1-serine metal complex it is found that: (*a*) precipitation does not occur; (*b*) the metal is not found in the ether phase (Table 1). The ATP effect might occur owing to the formation of co-ordination complexes between metal and ATP with no metal being available for the phospholipid. However, titration data as shown in Fig. 1, taken in conjunction with solubility characteristics (Table 1), suggest that a ternary metal–ATP–PS co-ordination complex forms. More explicitly, when PS is added to Fe(III) ATP solutions, Cu(II) ATP solutions or additional protons are released. This could occur owing to the formation of a ternary complex or the displacement of ATP by the phospholipid. If the latter were the case,

however, due to alterations in solubility, one would expect to find significant quantities of Fe(III) in the ether phase (Table 1). That this does not occur supports the argument for ternary complex formation between Fe(III), ATP and phospholipids. Moreover, in titrations (not shown) of metal, PS, and ATP in aqueous solutions (0·1 N KCl, 0·05 N NaCl) a precipitate did not form whereas precipitation did occur if only metal and the phospholipid were present. Owing to the physical characteristics of Mg(II), evidence for ternary complex formation from Mg(II) titration data is less firm than in the case of Fe(III) and Cu (II). However, if magnesium were present in greater than equimolar concentrations, as might occur in biological systems, then by the law of mass action increased complex formation would occur, as was shown for the case of Mg (II) and NE in an earlier report[1]. The increased solubility of the metal-phosphatidyl-1-serine-ATP complex in water and decreased solubility in ether is thought to be a function of the quadrivalent ATP. The presence of a ternary complex is not too surprising as ATP is known to form a chelate structure M(ATP)[8–12] and the serine group could easily form a 5-membered chelate ring utilizing the remaining co-ordination positions of the metal. In terms of overall electrostatic charge, alterations in solubility may be understood.

Table 1

Metal	5·00 μM phosphatidyl-1-serine: Without ATP	5·00 μM phosphatidyl-1-serine: 6·25 μM ATP	5·00 μM phosphatidyl inositide: Without ATP	5·00 μM phosphatidyl inositide: 6·25 μM ATP	No phospholipid
6·25 μM $CuCl_2$	2·32 ± 0·64	0·016 ± 0·002	1·66 ± 0·021	0·071 ± 0·005	0
6·25 μM $FeCl_3$	0·68 ± 0·32	0·025 ± 0·002	0·88 ± 0·76	0	0
6·25 μM $MgCl_2$	0·59 ± 0·18	0·03 ± 0	1·16 ± 0·29	0·087 ± 0·005	0

Values refer to μM metal/3 ml. of ether ± *S.E.M.* The No. for all metals + phospholipid is 3 and for all metals + phospholipid + ATP is 2.

As can be seen from Table 1, in the presence of phosphatidyl inositide metal is found in the ether phase in increased amounts. Titration results (Fig. 2) suggest the presence of a metal-phosphatidyl inositide co-ordination complex. It is also noted that, as before, metal incorporation into the ether phase is essentially reduced to zero in the presence of ATP. Evidence from titration data (Fig. 2) and solubility characteristics (Table 1) suggest the formation of a ternary co-ordination structure between metal, ATP, and phosphatidyl inositide. The dissociation characteristics of phosphatidyl inositide are such that the titration data with this phospholipid more clearly suggest

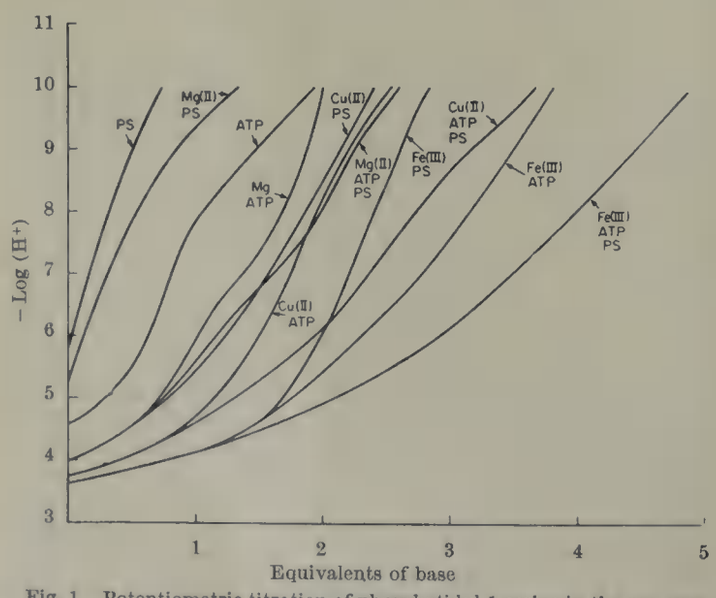

Fig. 1. Potentiometric titration of phosphatidyl-1-serine in the presence of various combinations of ATP, Mg(II) and Cu(II) (equimolar, 0·01 mmoles in 100 ml. water: isopropanol: *n*-butanol, 2 : 3 : 2) with carbonate-free 0·1 N KOH. Samples containing Fe(III) were equimolar with the other components at 0·01 mmoles

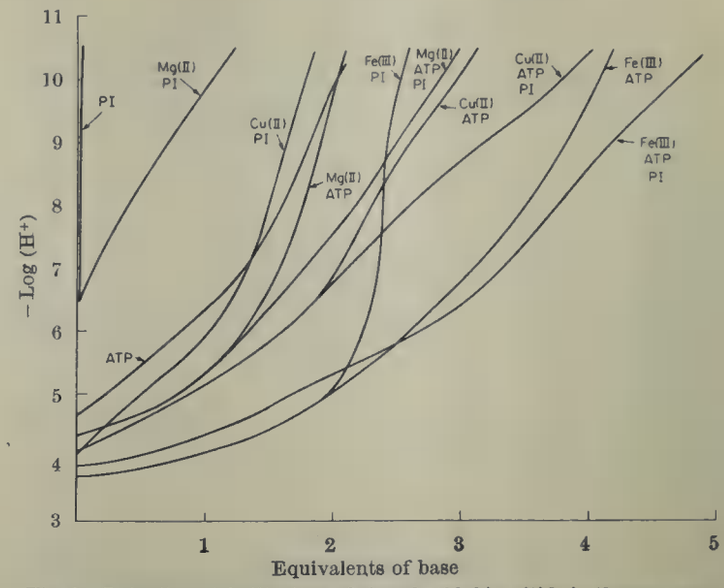

Fig. 2. Potentiometric titration of phosphatidyl inositide in the presence of various combinations of ATP, Mg(II), Cu(II) and Fe(III) (equimolar, 0·01 mmoles in 100 ml. water : isopropanol : *n*-butanol, 2 : 3 : 2) with carbonate-free 0·1 N KOH

ternary complex formation for Cu(II) and Mg(II) as well as Fe(III).

Although the co-ordination structures of these phospholipid-metal complexes are not clear it does seem that the structures are sufficiently stable in the presence of water to alter the solubility characteristics of the metal-phospholipid complex.

The two phospholipids thus far mentioned were chosen because of differences in their polar groups as well as being naturally occurring membrane constituents. It is not felt, however, that the change in solubility characteristics induced by metal complexes need be limited to these two. Any molecule which can co-ordinate stably with metal with an alteration in overall electronic structure would be expected to exhibit the characteristics shown by the two phospholipids examined. This phenomenon is well known, namely, precipitation of amino-acids by Cu(II), the inner complex salt of Werner[13].

To be certain that the results and theory constructed from them would be applicable to the central nervous system, lipids were extracted from a pellet of the synaptic fraction of an ultra-centrifugate of dissected brain. As a control the upper phase as defined by Folch, Lees and Sloane-Stanley[7], which contains predominantly water-soluble material, was used. The ability of this lipid extract to increase the incorporation of metals into the ether phase and a prevention of this by the presence of ATP are shown in Table 2. This was not found with upper phase material, which is taken as suggesting that lipids from biological material, synapses, will co-ordinate with metals and alter their electronic structure. Further, in the light of preceding data it seems reasonable to assume that ternary complexes form between synaptic lipids, metal, and ATP.

It is of interest that Folch, Lees, and Sloane-Stanley[7] find that $MgCl_2$ 0·003 N, $CaCl_2$ 0·003 N, NaCl 0·05 N, KCl 0·05 N are able to alter the distribution of lipids between the upper and lower phases, that is, the metals increase the yield of lipids in the lower, chloroform phase. Wolman and Wiener[14] report that when myelins

Table 2

	Lipid extract of synaptosomes: Without ATP	Lipid extract of synaptosomes: 6·25 μM ATP	Upper phase extract of synaptosomes	No. extract
6·25 μM $CuCl_2$	1·17 ± 0·126	0·012 ± 0·001	0	0
6·25 μM $FeCl_3$	0·61 ± 0·022	0·035	0·023 ± 0·0071	0
6·25 μM $MgCl_2$	0·086 ± 0·013	0·025 ± 0·005	0	0

Values are expressed as μM metal/3 c.c. ether/mg extract (dry weight) ± *S.E.M.*; No. = 4 (approximately 1 mg dry wt. of lipid extract was used per tube; approximately 4 mg dry wt. of 'upper phase' extract was used per tube). 11 mg of the lipid extract of synaptosome before addition of metals was found to contain 0·005 μM Cu, 0·0048 μM Fe, and 0·037 μM Mg.

are homogenized in 0·5 M calcium chloride an aqueous extraction yields only small amounts of lipid. They interpret this as indicating that the Ca^{++} has induced a transition of phospholipids to a water-in-oil pattern (Fig. 3). West[15] also notes the ability of metals to induce this transition. Others have suggested a role for membrane phospholipids in ion transport[16,17]. These investigators, however, have used the concept of electrostatic, attractive forces to explain the effect of metals on polar lipids. It is suggested here that the use of the theory and facts of co-ordination chemistry is more appropriate for the understanding of interactions between membrane phospholipids and metals as this body of knowledge: (*a*) aids in the understanding of differences in behaviour of various cations; (*b*) allows the introduction of steric considerations; (*c*) suggests that relatively stable complex formation (such as chelation) may occur between membranes and metals with an alteration in their solubility characteristics; (*d*) raises the possibility of ternary complex formation between a membrane constituent, metal, and biologically active molecules, namely, ATP amino-acids, metal activated enzymes. This latter point is of particular importance because of the well-known relationship between ATP enzymes and the maintenance of membrane function.

The results so far presented may be interpreted as follows. Phospholipids alone dispersed in aqueous systems are oriented with their polar heads towards the aqueous phase (in either lamellar sheets or globular arrays). In the presence of metals co-ordination complexes between the metal and phospholipids form which alter the electronic structure of the complex in such a way that a portion of the phospholipid dispersion assumes a water-in-oil configuration (Fig. 3) and hence the increase in ether solubility of the metal–phospholipid complex. If one adds ATP, however, the situation is quite different. Owing to either the formation of ternary complexes or the functional removal of metal by ATP the phospholipid dispersions are shifted towards a predominantly oil-in-water pattern. In this system metals and ATP have a role as important modulators of the configurational array assumed by phospholipids in aqueous systems.

The significance of this for biological membranes is suggested to be as follows. Metal–ATP–phospholipid complexes can be seen as forming lamellar sheets in agreement with the most generally accepted model depicting the cellular membrane[18–22] as composed of two unimolecular lipoprotein leaflets having the phospholipid molecules so oriented that the hydrocarbon tails point toward each other. However, if the ATP is removed, by ATPase, for example, the electronic structure of the metal–phospholipid complex will favour the development of a quite different configuration. Replacement of the ATP, by a metabolic source, will lead to a resumption of the usually depicted lamellar configuration. In the understanding of membrane function in nerve, some mechanism, whereby rapid reversible alterations in membrane structure occur, is necessary. How such a mechanism might operate in a biological system, such as a synapse, is presented in Fig. 4. If such a model is correct, the following can be predicted.

(1) Whether on the inner or outer surface of the cellular membrane, metals will tend to induce configurations of the water-in-oil type, that is, to produce a mosaic of water-filled channels or pores lined with charges. The dimensions of these pores may easily[23] be of sufficient size to permit the passage of ions and small molecules across the membranes. Larger molecules which can co-ordinate with the sterically available metals may cross the membrane in an exchange reaction.

(2) The pores in this model have fixed charges and varying dimensions of a type dependent on the particular phospholipid and metal involved. They could thus assume the properties of permselective membranes[24]. In this respect it is of interest that copper converts the frog skin membrane into a structure which becomes selectively impermeable to chloride ions[25].

(3) It is generally agreed that ATP is quite important in membrane function and is known to be present in the *milieu interieur* of the cell and synapse; for example, Nyman and Whittaker[26] have shown that a stable ATP fraction which is probably only about 10 per cent of that found *in vivo* is present in synapses in a molar concentration some 200 times greater than serotonin. From our results it is suggested that complexes between ATP–metal–phospholipids would tend to maintain the lamellar arrangement, and as the metabolic source of ATP is in the interior of the cell this will lead to different structural configurations between the inner and outer surfaces of the membrane, that is, there are many less water-in-oil configurations on the inner surface. This would mean that in the presence of ATP the energy barrier to passage of small ions such as sodium or potassium and larger molecules in either direction across the internal interface would be relatively large, that is, selective ion concentrations and hence potential gradients would tend to be maintained as well as there being a barrier to the passage of polar molecules.

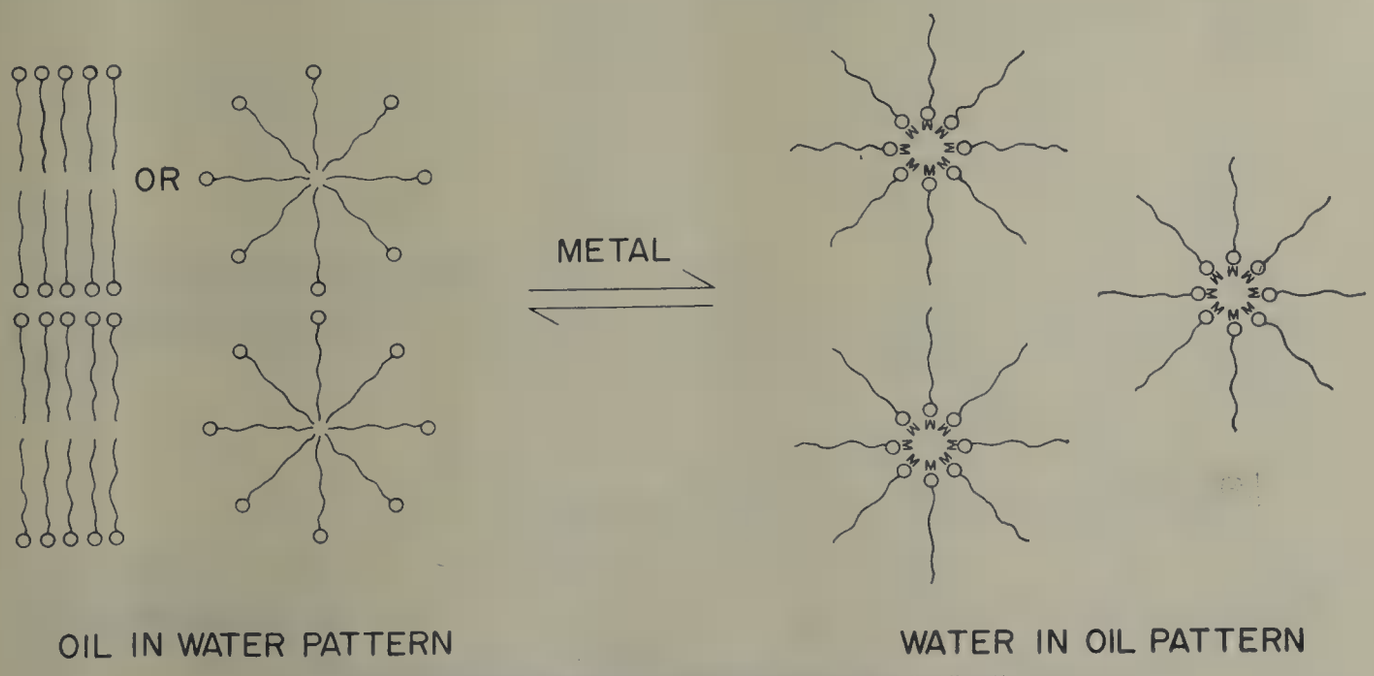

Fig. 3. Schematic representation of oil-in-water and water-in-oil patterns

(4) Removal of ATP from the ATP–metal phospholipid complex as by ATPase, which may be intimately associated with membrane function in nerve[27], would result in the internal surface of the membrane spontaneously assuming a water-in-oil configuration, that is, one would now have a mosaic of charged pores both on the inner and outer surfaces of the membrane with consequent fluxes of water and ions across the membrane in both directions. Two especially interesting features predicted from this are: (*a*) the loss of the membrane potential due to Na^+ and K^+ exchange; (*b*) a change in membrane resistance.

(5) Replacement of ATP on the metal–phospholipid groups would result in a resumption of the resting structure, that is, one without pores on the inner surface and an energy barrier to movement of ions and larger molecules across it. Without a steady supply of ATP this resumption would not occur and the membrane would progressively, functionally deteriorate.

(6) Some miscellaneous features of the model to be noted are as follows: Luzzati and Husson[23] have shown that the phospholipids extracted from brain, at body temperature, are not in a coagel but a liquid crystalline state, that is, at 37° the hydrocarbon tails have melted. As such the energy barrier of the hydrocarbon tails to transitions between the two configurations would be less than with a coagel. Furthermore, they have found by X-ray diffraction data that such water-in-oil to oil-in-water transitions can spontaneously occur in phospholipid–aqueous dispersions depending on concentration. As such, counter forces in either direction could be quite determinant as to the configuration assumed.

It is of interest that Baker, Hodgkins and Meves[28] have raised the possibility of there being fixed negative charges on the inner surface of the membrane which they relate to ionized phospholipid and carboxyl groups. Quadrivalent ATP might significantly contribute to such fixed negative groups.

Other, less metabolically active, molecules might also function as ATP in the maintenance of the oil-in-water configuration and would tend to stabilize the membrane in this form. Attention has been focused on ATP because of the importance of its production and dephosphorylation for normal membrane function.

Data and theory bearing on a mechanism whereby a neurotransmitter, norepinephrine, might be released from the pre-synaptic terminal are now presented. In the preceding article it was noted that metals found in the synaptosomes and synaptic vesicles tended to remain with the particulate fractions and not enter the supernatant. Further, evidence for the formation of metal–NE chelate structures and their importance in storage pools within synaptic vesicles and synaptosomes was discussed. In the light of results presented in the earlier portion of this article, it seems possible that ternary complexes might form between membrane phospholipids, metals, and NE or even quaternary complexes with ATP where the co-ordination number of the metal is 6 or more.

Titration curves (Fig. 5) suggest that such ternary complexes do occur between phosphatidyl-l-serine, metals and *d*,*l*-norepinephrine.

Solubility characteristics of metals and NE in the presence of two phospholipids are presented in Table 3. It is noted that in the presence of metals and phospholipids the quantities of metal in the ether phase and the partition coefficients of NE are markedly altered. A NE–metal–phospholipid complex is thus seen to have altered solubility characteristics such that a polar NE molecule becomes more soluble in an organic solvent. Phrased differently, the energy barrier to the movement of NE into an organic solvent is reduced by complexing. This might be of significance in understanding a mechanism

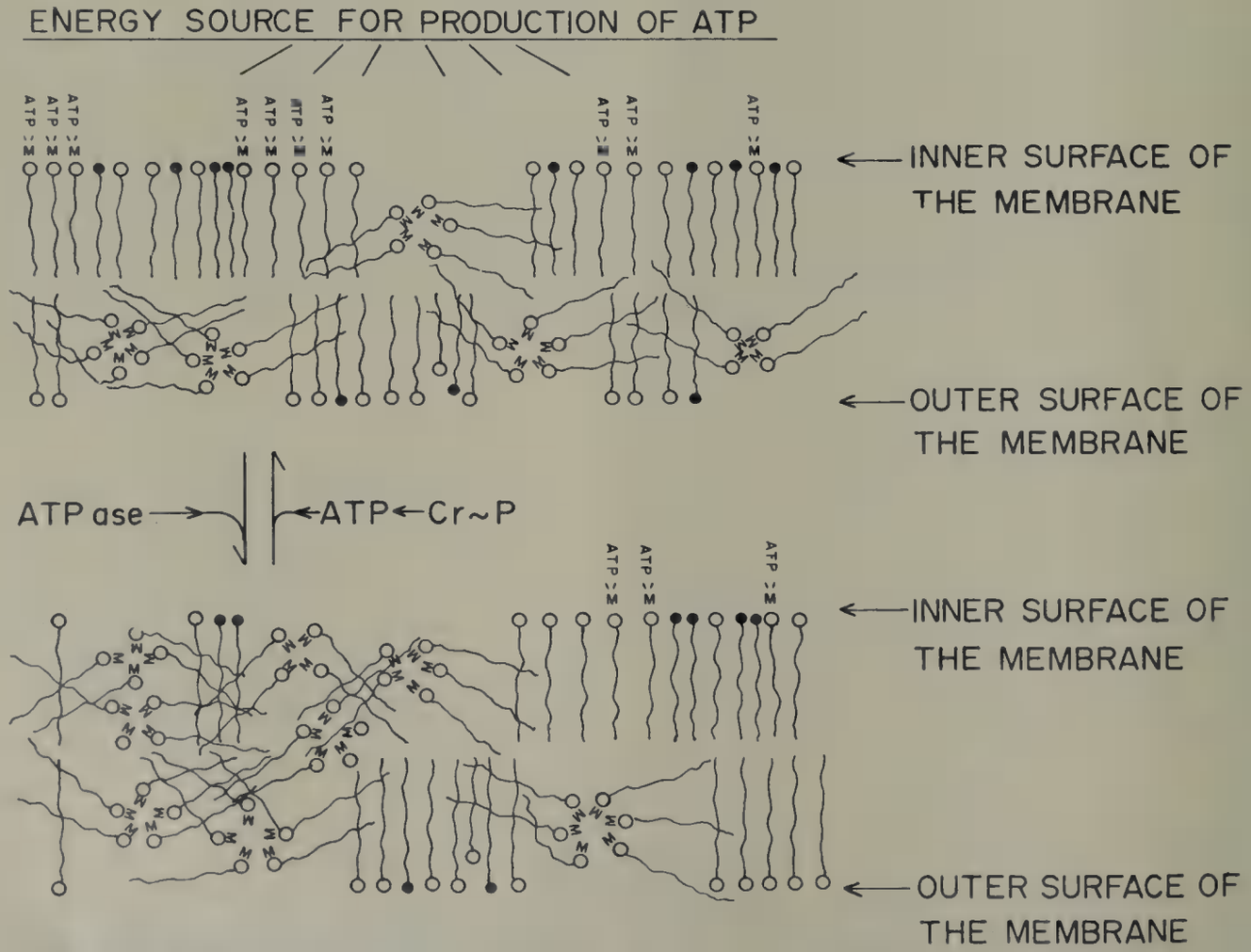
ENERGY SOURCE FOR PRODUCTION OF ATP
INNER SURFACE OF THE MEMBRANE
OUTER SURFACE OF THE MEMBRANE
ATPase
ATP←Cr~P
INNER SURFACE OF THE MEMBRANE
OUTER SURFACE OF THE MEMBRANE

Fig. 4. Schematic representation of suggested cell membrane transitions as outlined in the text. (The letter *M* represents metals. Filled black circles indicate membrane components which form relatively unstable co-ordination complexes)

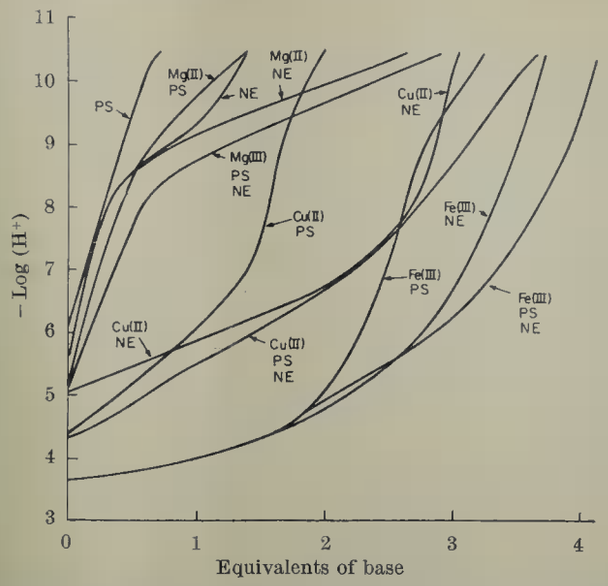

Fig. 5. Potentiometric titration of phosphatidyl-1-serine in the presence of NE, Mg(II), Cu(II), and Fe(III) (equimolar, 0·01 mmoles in 100 ml. water : isopropanol : *n*-butanol, 2 : 3 : 2) with carbonate-free 0·1 N KOH

whereby a polar molecule such as NE moves across the potential energy barrier of the lipid membrane.

In Table 4 data on the effects of salt concentrations on metal concentrations in the ether phase and NE partition coefficients are presented. It is noted that there is a marked effect in general but that this is independent of the ion species, that is, sodium and potassium do not cause differential effects. The changes noted are felt to be a function of the salts increasing the polarity of the aqueous phase with a consequent accentuation of solubility characteristics. This raises the possibility that in biological systems the solubility changes reported throughout this article would be accentuated.

In Table 5 it is noted that increasing concentrations of ATP progressively diminish the amount of metals found in ether as well as decreasing the partition coefficients of NE. Possible co-ordination complexes by which this might occur are several, but the important point is that the presence of ATP could serve as an important modulator of the amount of NE which is able to surmount the energy barrier represented by the membrane.

Finally, similar effects of lipid extracts of synaptosomes and upper phase extracts are presented in Table 6. It is noted again that the presence of the lipids increases both partition coefficients and metal content of the ether phase and that this is inhibited by ATP.

From these results the following hypothesis regarding synaptic membrane function and NE is suggested. In the resting membrane the presence of ATP increases the energy barrier to the movement of NE across the internal surface of the membrane. If ATP is removed, as by an ATPase, either (*a*) unfilled co-ordination positions on the metal–phospholipid become available to NE, or (*b*) the removal of ATP from a quaternary complex occurs and a ternary phospholipid–metal–NE complex remains. In either case, the electronic structure of this ternary complex is such that its solubility in the aqueous phase is decreased, whereas in the lipid phase (the cell membrane)

Table 3

Metal content is expressed as μM/3 c.c. ether ± *S.E.M.* (No. = 3 to 5; *N.D.* means not done)

Metal concentration		No phospholipid; no NE	5·00 μM phosphatidyl-1-serine + 6·25 μM NE	5·00 μM phosphatidyl inositide + 6·25 μM NE	6·25 μM NE
0	NE partition coefficient	—	0·0037 ± 0·0003	0·007 ± 0·0001	0·0103 ± 0·0016
	Metal content	0	—	—	—
6·25 μM $CuCl_2$	NE partition coefficient	—	0·11 ± 0·030	0·151 ± 0·041	< 0·001
	Metal content	0	1·6 ± 0·161	2·27 ± 0·276	< 0·0009
6·25 μM $MgCl_2$	NE partition coefficient	—	0·035 ± 0·004	*N.D.*	0·0127 ± 0·0001
	Metal content	0	0·879 ± 0·080	*N.D.*	0·0264 ± 0·0072

Table 4

	NaCl = 0 KCl = 0		NaCl = 100 m molar KCl = 20 m molar		NaCl = 100 m molar KCl = 100 m molar		NaCl = 20 m molar KCl = 100 m molar	
	Partition coefficient (NE)	Mg (μM/3 ml.)	Partition coefficient (NE)	Mg (μM/3 ml.)	Partition coefficient (NE)	Mg (μM/3 ml.)	Partition coefficient (NE)	Mg (μM/3 ml.)
NE 6·25 μM (No. = 4)	0·0111 ± 0·0017	0·014 ± 0·022	0·0137 ± 0·0024	0	0·0138 ± 0·0024	0·012 ± 0·03	0·014 ± 0·0009	0·031 ± 0·013
NE 6·25 μM $MgCl_2$ 6·25 μM (No. = 4)	0·0159 ± 0·0001	0·021 ± 0·002	0·0135 ± 0·0024	0·023 ± 0·002	0·015 ± 0·0002	0·021 ± 0·003	0·0106 ± 0·0003	0·023 ± 0·003
NE 6·25 μM PS 5·0 μM (No. = 2)	0·0036 ± 0·0007	0·033 ± 0·011 ± 0·0036	0·0098 ± 0·001	0·021 ± 0·025 ± 0·0084	0·01 ± 0·0007	0·009 ± 0·005	0·0764 ± 0·0071	0
NE 6·25 μM $MgCl_2$ 6·25 μM PS 5·0 μM (No. = 6)	0·041 ± 0·002	0·762 ± 0·033 ± 0·011	0·064 ± 0·0033	0·906 ± 0·063	0·0653 ± 0·002	0·888 ± 0·057	0·0596 ± 0·0037	0·849 ± 0·027

Table 5

Magnesium content is expressed as μM/3 ml. ether ± *S.E.M.* The No. in all cases is 5

		ATP 0	ATP 1·25 μM	ATP 2·5 μM	ATP 5·0 μM	ATP 10·0 μM
5·0 μM phosphatidyl-1-serine + 6·25 μM NE + 6·25 μM $MgCl_2$	Partition coefficient NE	0·042 ± 0·002	0·0351 ± 0·0018	0·0251 ± 0·0035	0·0107 ± 0·002	0·0036 ± 0·0005
	Magnesium content	0·639 ± 0·051	0·537 ± 0·018	0·411 ± 0·09	0·156 ± 0·036	0·021 ± 0·0033

its solubility is increased. The net result is an increase in movement of NE across the cell membrane. The mechanism of movement of the NE molecule is not clear, that is, it might occur simply as an exchange reaction or be a function of the development of a water-in-oil pattern which sterically would place the NE molecule in the membrane pore as outlined in an earlier part of this article. Displacement of NE by ATP (from a metabolic source *in vivo* or an exogenous source *in vitro*) would bring about a resumption of the usual resting structure. Note that by this mechanism the membrane ATP will tend to prevent loss of NE from the vesicle and that a steady supply of ATP is necessary to prevent the vesicle from functionally deteriorating.

Table 6

		Lipid extract of synaptosomes + 6·25 μM NE		Upper phase synaptosomes + 6·25 μM NE
		Without ATP	6·25 μM ATP	
6·25 μM $CuCl_2$	Partition coefficient NE	0·046 ± 0·017	0·003 ± 0·001	All values < 0·001
	Copper content	0·320 ± 0·113	0·0155 ± 0·0027	0
No copper	Partition coefficient NE	0·0027 ± 0·0014	—	All values < 0·001

Metal content is expressed as μM Cu/3 c.c. ether/mg extract (dry wt.) ± *S.E.M.* with No. = 5 or 6. Approximately 1 mg (dry wt.) of lipid extract was used per tube; approximately 4 mg (dry wt.) of upper phase extract was used/tube.

The report[29] that cold NE can be taken up against a gradient by granules known to be rich in this amine can be satisfactorily explained by the presence of metals within the granules[1]. In addition, reports[29,30] suggest magnesium and ATP are necessary for uptake of NE or E by chromaffin granules. The great bulk of the data reported by these investigators involved rate curves for the appearance of radioisotopically labelled catecholamines within the granules. As such, any factor causing an increase in endogenous pool size would lead to a less rapid exit of the isotopically labelled molecule and hence higher c.p.m. As noted before[1], we feel that there is a true uptake, against a gradient, owing to the presence of metals within the granules, but that in addition the presence of magnesium and ATP diminishes the rate of outflow of NE from the interior of the vesicle for reasons which follow from the suggested role of ATP and metal in membrane function and NE transport. The combination of true uptake against a gradient due to metal within the vesicle and diminished outflow due to magnesium–ATP then leads to greater amounts of radioactivity being found within the granule.

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ACETOHYDROXAMATE: BACTERIAL UREASE INHIBITOR WITH THERAPEUTIC POTENTIAL IN HYPERAMMONAEMIC STATES

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DURING investigations demonstrating that the antileukaemic agent, hydroxyurea, is both a substrate and an inhibitor of jackbean urease[1–3], acetohydroxamic acid (AHA) was used as an analogue of hydroxyurea. AHA was found to be an irreversible, non-competitive urease inhibitor with a rate constant of 10^3 M^{-1} min^{-1} at 25° C, that is, 1 mmole AHA produced complete urease inactivation in 3–4 min[3]. Since the compound has little toxicity for animals[4] and has been shown to exert no inhibitory effects on a variety of other enzyme systems[5], it was examined for possible clinical application in treating hyperammonaemic states by reducing the contribution to blood ammonia by intestinal urease activity. The need for such an agent has been expressed elsewhere[6,7]. The work described here establishes the inhibitory effect of AHA on bacterial urease *in vitro* and *in vivo*, and reports the effect of the compound in a single patient with liver disease.

Following preparation and purification of AHA[2], a preliminary assessment of excretion and acute toxicity was made. Intraperitoneal administration of a single dose of 900 mg/kg to each of three adult rats resulted in no gross clinical disturbances over a one-month observation period, and the full dose was recovered (98, 100 and 110 per cent) in the urine excreted during the first 24 h, as determined by ferric chloride assay[2]. Although this assay method is not definitive (it would not, for example, discriminate AHA from other hydroxamic acids that might occur as biological derivatives), the results suggest that AHA is rapidly and, in contrast

to hydroxyurea[8], completely excreted. Five mmoles AHA (375 μg/ml.) in trypticase soy broth produced no growth inhibition in tetracycline-sensitive strains (0·2–25 μg/ml.) of *Staphylococcus aureus*, β-haemolytic *Streptococcus* Group *A*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Brucella* sp., and *Bordetella* sp. Unlike hydroxyurea, which regularly inhibits the growth of *Pseudomonas* organisms[9], AHA thus appears to be devoid of antibacterial activity despite its very similar chemical structure.

Urease inhibition by AHA was evaluated for the same organisms (excepting the *Streptococcus* and *E. coli*) in standard bacteriological media[10], which contain a highly buffered solution of urea at a concentration approximating that of urine. Gradation of the colour change of the phenol red indicator produced by the alkaline ammonium carbonate liberated from urea serves as a convenient index of the degree of urea decomposition. In all six species, AHA, at concentrations of 1 mmole or less, significantly inhibited the ureolytic activity. Fig. 1 illustrates the data for *Klebsiella* sp. and *Proteus mirabilis*.

In one experiment a duplicate set of cultures was used, omitting the indicator; aliquots were acidified, Seitz-filtered, diluted and analysed directly for urea[11]. The inhibition is more impressively demonstrated by this method, and the correlation of urea decline with indicator changes is evident (Fig. 1). Since saturating levels of urea are present in these experiments, the non-competitive nature of AHA inhibition is further substantiated, and suggests that the action of the drug would not be compromised by even the high urea concentrations of urine. The ultimate appearance of ureolytic activity in the media containing AHA may simply reflect the unchecked bacterial growth.

AHA inhibition of bacterial urease activity *in vivo* was assessed in adult rats by the use of ^{14}C-urea[12]. Twenty-five μc. of the isotope was injected subcutaneously immediately after intraperitoneal injection of AHA. The animal was placed at once in a metabolic chamber[13] and

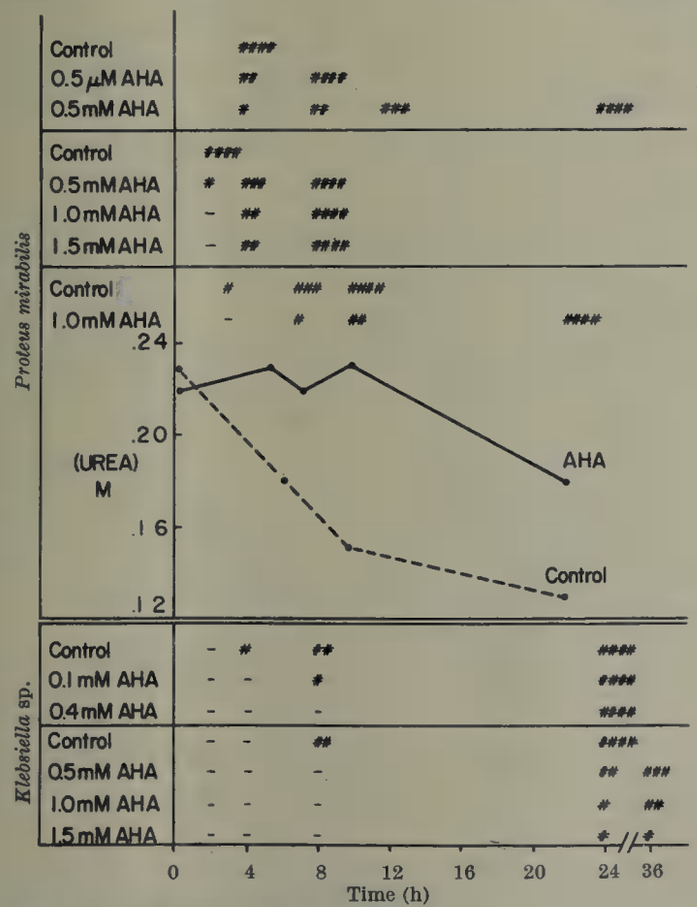

Fig. 1. Inhibition of bacterial ureolytic activity by AHA in urea broth cultures. The colour changes of phenol red indicated produced by alkaline ammonium carbonate liberated from urea are graded 1 to 4+. Direct measurement of urea was also performed. See text for details

Table 1. Percentage of ^{14}C-urea expired at $^{14}CO_2$ in Rats given a 25-μc. Dose Subcutaneously immediately after Intraperitoneal Administration of AHA

The figures in parentheses denote percentage inhibition during the individual collection periods relative to the control values

Expt. sequence	Rat wt. (g)	AHA (mg/kg IP)	Cumulative % administered ^{14}C-urea expired as $^{14}CO_2$ (h) 1	2	3	4
Controls:						
4	295	0	2·16	6·7	10·3	12·6
6	230	Normal saline		6·6		
1	240	0			9·0	
Treated:						
2	260	100			4·7 (51)	
5	307	50	0·66 (69·5)	1·9 (72·5)	3·2 (63)	4·2 (58)
3	220	20			2·7 (72)	
7	280	5		4·26 (36)		
8	260	10		3·6 (45)		
		Tetra-cycline				

the expired carbon dioxide was collected in 4 N NaOH, aliquots of which were counted directly in a liquid scintillation spectrometer by the two-phase anthracene method of Steinberg[14]. The radioactivity of expired carbon dioxide amounted to about 3 per cent of the administered dose of urea per h in the control animals (Table 1); it was reproducible, and was consistent with the values obtained by others in mice[15–17]. AHA, at doses of 20–50 mg/kg, resulted in a 70 per cent inhibition of urea decomposition during the first 3 h. In the experiment involving hourly collections, the inhibition seems to begin to decline in the third and fourth hours. The lesser degree of inhibition occurring when a large dose of tetracycline is substituted for AHA may be reasonably presumed to be due to the different modes of action of the two drugs. AHA directly inhibits urease without any demonstrable concomitant antibacterial activity (which, incidentally, suggests that under the particular conditions involved, urease is functioning as a 'scavenger' enzyme rather than in any essential metabolic sequence). On the other hand, the antibiotics, although capable of completely suppressing *in vivo* urease activity, do so not by any direct action on the enzyme, but by suppressing bacterial growth[17,18]. The short time-interval used in these experiments, therefore, does not permit the indirect action of tetracycline to reach maximum effect. Interestingly, complete suppression of ureolysis by AHA was not attained even at high dose levels. While the reason for this is not clear, it may possibly represent persistent gastric urease activity owing to failure of the enzyme-inhibitor complex to form under acid conditions[5].

Although urine collections over these brief periods are subject to considerable error, urinary radioactivity in these experiments approximated 13 per cent of the injected dose per hour. The fraction of acid-labile radioactivity was within the error of determination for both the ^{14}C-urea reagent (1 per cent) and all urine specimens (5 per cent).

While AHA seems clearly to be an effective inhibitor of bacterial urease, a more critical question concerns the feasibility of using this agent as a drug to lower elevated blood ammonia-levels. Since the compound is not generally available, and chronic toxicity examinations will probably consume large quantities of it, a preliminary trial of the compound was made in a single patient to assist in deciding whether a full-scale investigation should be undertaken[19]. The patient, a 60-year-old male with chronic myelogenous leukaemia and leukaemic infiltration of the liver, had received an end-to-side portocaval shunt nine months before to relieve jaundice and hepatic coma consequent to portal vein thrombosis. At the time of this investigation, his haematological status was stable, despite elevated platelet and white cell counts; his liver functions were entirely normal save for elevated blood ammonia-levels. The patient was maintained at bed-rest

and fasting until 2 p.m. each examination day and received the drug orally in capsule form, with half of each dose being enteric-coated. Antecubital vein blood was withdrawn without stasis via an indwelling Cournand needle, and blood ammonia determinations were performed immediately, in duplicate, by the method of Seligson and Hirahara[20]. The results are shown in Fig. 2; the decline and return of blood ammonia-level following the larger dose of AHA, while certainly suggestive, are not definitive because of the wide physiological and/or measurement fluctuation apparent during the control day. It would seem unlikely that the absence of a striking decline in ammonia-level is due to inactivity of oral AHA in man, since the drug, in this instance, is acting, not on host enzymes, but on those of bacteria in the alimentary canal. It seems more reasonable to presume that, at least in this particular case, bacterial urease is not playing a decisive role in maintaining elevated blood ammonia-levels. While it is clear that urea nitrogen can be used in anabolic processes[21,22] and that the administration of urease and/or urea can produce ammonia intoxication[7,23,24], the fraction of total blood ammonia-levels in man attributable to urease, as compared to other bacterial and host ammonia-generating enzyme systems, has never been evaluated. A combination investigation using ^{14}C-urea, a specific urease inhibitor such as AHA, and antibiotics might provide an answer to this question. The limiting factor in such an approach, at present, is the variability in blood ammonia determinations[25]. In the patient examined, for example, if the maximum decline in blood ammonia on day five was taken literally as due to AHA, then, assuming 70 per cent urease inhibition at this dose, the fraction of the stable blood ammonia contributed by this 'exogenous' enzyme would be an impressive 35 per cent. Yet this degree of decline in blood ammonia is still not a conclusive change.

No symptoms attended the administration of AHA to this patient, and no effects on the haemogram or hepato-renal function tests were observed during a five-day hospital observation period. Since the toxic effects of chemically related compounds occur and subside quickly in relation to drug administration and withdrawal[26,27], more extensive trials of AHA in man would appear practicable.

The present data indicate that further investigation of AHA to define its clinical potential is warranted. Since urease inhibition is a general feature of hydroxamic acids[5], it is, of course, possible that another of these compounds may ultimately be found to be more suitable. AHA was selected by us because of information available regarding its specificity and low toxicity. Like AHA, hydroxyurea is also an irreversible urease inhibitor, but, unlike AHA, a residual fraction of enzyme activity does persist in its presence[3]. Hydroxyurea is decomposed by urease with the liberation of ammonia, while AHA is inactive as a substrate[1,2]. Hydroxyurea produces extensive chromosomal breakage in cultures of hamster and mouse embryo cells[28] and of normal human leucocytes[29], while AHA is inactive in this regard[29]. The problem of megaloblastosis attending hydroxyurea treatment[26,30,31] may yet turn up on extensive toxicity investigations with AHA, but on the basis of available knowledge, the latter compound would seem to offer a definite superiority as a urease inhibitor in man.

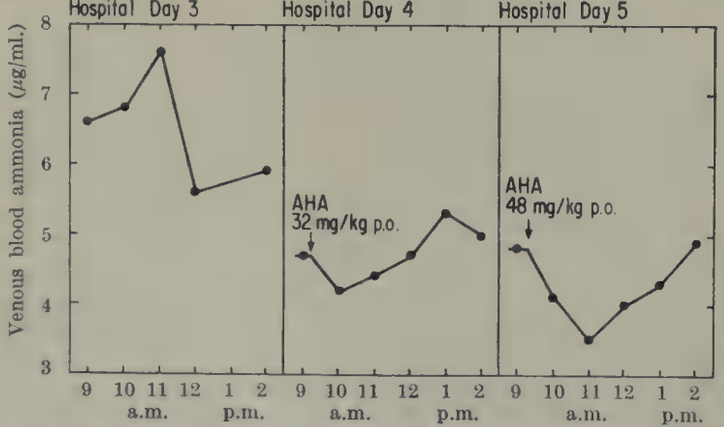

Fig. 2. Venous blood ammonia-levels in a human patient during a control day and following two oral (dose) trials of AHA. The patient was maintained fasting and at bed-rest during each study period. Normal venous values with this method are 0·7–2·0 μg NH_3/ml. blood

AHA appears to possess most of the characteristics desirable in an ideal anti-urease drug. (*a*) Its inhibition is specific, complete, and irreversible and occurs via complex formation rather than by oxidative phenomena[3,5]. (*b*) It can be expected to be free of allergenic dangers attending the use of urease antibodies[6]. (*c*) Its action would complement, rather than mimic, that of antibiotics. In addition to the most evident applicable clinical situation, hepatic failure, it is possible that hyperammonaemia may play a part in the acute morbidity of certain septicaemias, consequent to the urease activity present in many bacterial pathogens[32] in combination with hepatic anoxaemia. If so, a urease inhibitor might be of value in the initial critical phase of treatment, until antibiotic control of bacterial growth has been attained.

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RAPIDLY LABELLED SYNTHESIS OF RIBONUCLEIC ACID IN EHRLICH ASCITES C-2 TUMOUR CELLS TREATED WITH ACTINOMYCIN D

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ACTINOMYCIN *D* markedly inhibits DNA-dependent RNA polymerase, and inhibits DNA-primed RNA synthesis *in vitro* and *in vivo*[1,2]. This biological activity is correlated with its ability to form complexes with DNA-guanin residues[3]. There are more types of DNA-actinomycin *D* binding sites *in vitro*[4], and the synthesis of ribosomal and *s*-RNA *in vivo* is more sensitive[5] than the synthesis of DNA-like RNA[6]. A rapid turnover of messenger RNA[7] was originally thought to be a characteristic of this class of material, and may be defined, so far as is known at present, as a sequence-determining template in ribosomes for catalytic function in protein synthesis[8]. But in mammalian cells it is not clear how the transfer 'messenger' RNA of the labelled nuclear RNA is related to the stable cytoplasmic RNA[9]. When cells were incubated for 10 min with ^{14}C-adenine, then transferred to a non-radioactive medium containing actinomycin *D*, the pulse-labelled nuclear RNA underwent breakdown to acid-soluble end-products in the nucleus, no transfer of the pulse-labelled RNA from nucleus to cytoplasm could be detected[10–13]. On the other hand, when the cells were exposed to ^{14}C-adenine for 20 min and the radioactive precursor remained in the medium, after adding actinomycin *D*, experiments demonstrated the transfer of the labelled nuclear RNA to the cytoplasma[14]. The radioactivity may continue to be incorporated into both nuclear and cytoplasmic RNA at the highest rate 3–4 h after addition of the actinomycin *D* (ref. 14). In our work we found the same difference between 'normal' pulse-labelled RNA and the 'second' pulse-labelled RNA when the cells were treated for 4·5 h with actinomycin *D*. In contrast to the 'normal' pulse-labelled, the 'second' pulse-labelled RNA passed quickly into the cytoplasmic compartment, and also exhibited other differences.

Ehrlich ascites mouse tumour cells *C*-2 were grown as described previously[15]. A suspension of cells (500 mg dry weight) in Oerle medium (95 per cent O_2–5 per cent CO_2 at 37° C) were exposed for 10 min to uracil 2–^{14}C, 1 μc./ml. After labelling, the cells were washed with physiological saline and re-incubated in non-radioactive Oerle medium (5 μg/ml.) actinomycin *D*. Cells were taken after 15 min, and at intervals thereafter for 5 h. The respective fractions were denatured by acidification to 0·5 N PCA, washed once more with 0·4 N PCA, and neutralized with 1 N KOH using phenol red as an internal indicator. RNA was isolated with 10 per cent NaCl and subsequent ethanolic precipitation[17]. Following alkaline digestion with 0·4 N KOH for 17 h at 37° C the resulting ribomononucleotides were separated by column procedure on 'Dowex-1' $HCOO^-$ x–10 200–400 mesh[18]. The specific activities of RNA pyrimidines were determined in peak portions (0·1–0·2 μM) in a Nuclear Chicago assembly operating with a thin-window counter. The specific activities were expressed as c.p.m./μmole, based on the extinction coefficients given by Cohn[19]. The total error of the measurements by the parallel samples never exceeds ± 5 per cent.

During the 5 h more than 90 per cent of the radioactive pyrimidines is released from the total RNA to the acid-soluble end-products[10–13]. Residual radioactivity seems to come from heterogenous RNA in a stable form. In this system we have studied various nucleic acids after 'normal' pulse-labelling and 'second' pulse-labelling 4·5 h later. Aliquots of cells were enucleated by controlled stirring in a solution of 'Tween 80' (ref. 20) and the nuclear nucleic acids were separated with 1 M NaCl (ref. 21). These cytoplasmic fractions, 1 M NaCl nuclear extractable, and 1 M NaCl residual nuclear RNA, were treated for the specific activity of pyrimidines as described previously.

After the first pulse-labelling the earliest labelled RNA is extractable from nuclear RNAs with 1 M NaCl. In the

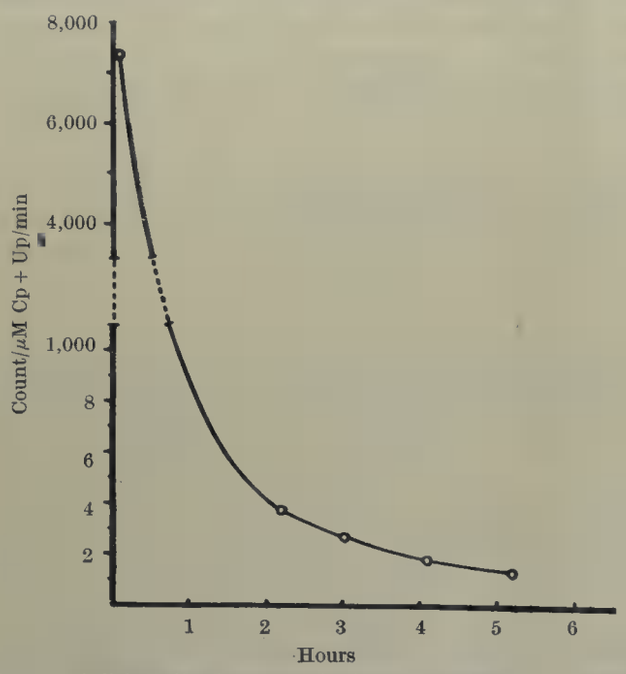

Fig. 1. Ehrlich ascites tumour cells *C*-2 were exposed to ^{14}C-uracil for 10 min. They were then washed and transferred at zero time to non-radioactive medium containing actinomycin *D* at a concentration of 5 μg/ml. Specific activities of total RNA pyrimidines (Cp Up) were determined for 6 h

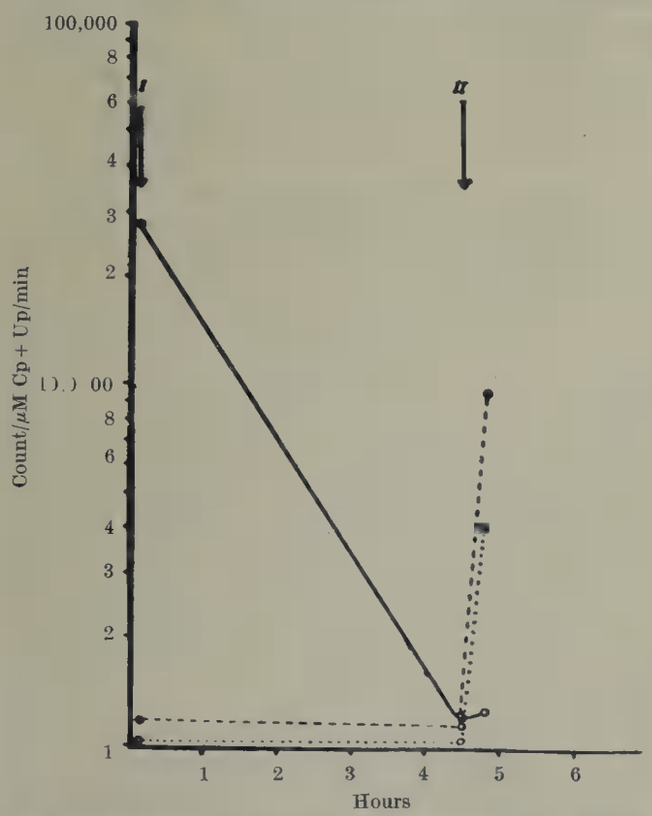

Fig. 2. Ehrlich ascites tumour cells *C*-2 were exposed to ^{14}C-uracil for 10 min. They were then washed and transferred at zero time to non-radioactive medium containing actinomycin *D* at a concentration of 5 μg/ml. for 4·5 h. The cells were washed and re-incubated for 10 min in ^{14}C-uracil. Specific activities of Cp Up were determined for . . . . cytoplasmic RNA, —— nuclear 1 M NaCl extractable RNA, - - - - nuclear 1 M NaCl non-extractable RNA

nuclear residual and cytoplasmic RNAs no radioactivity was determined. After the second pulse-labelling (DNA primed and dependent RNA synthesis was abolished with actinomycin *D* and 'normal' pulse-labelled RNA was degraded to an acid-soluble end-product), both the residual nuclear and cytoplasmic RNA were labelled. No radioactivity was found in the nuclear 1 M NaCl-extractable RNA.

Our results suggest the possible role of the 'normal' pulse-labelled RNA on the other biosynthetic routes. There is some difference between 'normal' pulse-labelled and 'second' pulse-labelled RNAs: 'normal' 10-min-labelled RNA is nuclear 1 M NaCl-extractable; 'second' 10-min-labelled RNA is not, and a possible interpretation is that it more quickly passed into the cytoplasmic compartment.

The 'second' pulse-labelled RNA seems to be of chromosomal origin; the nuclei treated with 'Tween 80' have no nucleoli[22]. These results point to an RNA-dependent biosynthetic route in Ehrlich ascites cells[23].

The present investigations demonstrate that there are more 'pulse'-labelled RNAs and demonstrate the transfer of messenger RNA from the nucleus to the cytoplasm.

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MICROMETHOD FOR MEASURING MICROBIAL SENSITIVITY TO DRUGS

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THREE methods are in use for testing sensitivity to drugs: (*a*) the accurate but laborious tube dilution method; (*b*) a method in which drugs are incorporated into ditch plates; (*c*) a method in which disks are impregnated with drugs. The last method, for its simplicity, is commonly used in clinical laboratories. In this method, the disk is placed on the surface of a sown, solid medium. The drug diffuses, and the presence of a zone, in which growth is inhibited, and the diameter of this zone, are considered to be indicative of the sensitivity of the organism towards the drug. However, it is difficult to relate the diameter of such a zone to results obtained in tube dilution tests. The diameter of a zone also represents a measure of the diffusibility of the drug. Penicillin, for example, has a much higher diffusibility than polymyxin, for which much higher disk contents are used[1]. Another shortcoming of the disk method is that no estimate of combined activity can be given. The zone reading indicates inhibition of organisms by the drug which diffuses through agar most rapidly[2].

In order to obtain a faster and more uniform diffusion, impregnated paper disks were placed at the bottom of 10-mm × 75-mm test tubes, and 0·5-ml. Baltimore Biological Laboratory soy agar was poured on them. Hereby the path which the drug had to travel was kept extremely short. After 1 h the agar was inoculated with 0·05 ml. of an 18-h-old culture of *Staphylococcus aureus*, type 81, standardized to contain 10^6 organisms/ml. (ref. 3). The results, however, were not consistent. When pouring the agar over the disk, a slightly raised agar rim forms. This rim apparently contains less drug than the plane surface of the agar. Colonies often developed on the rim, while growth was absent from the plane part of the agar.

Further experiments were carried out with streptomycin disks, containing 2, 5 and 10 μg, cut into halves and quarters. It was found that the degree of elution depends on the size of the exposed area. For example, one half of a 2 μg disk did not inhibit growth, while another half, cut into two quarters, did inhibit growth; one-half of a 5 μg disk did not inhibit growth, while growth was inhibited by a whole 2 μg disk.

We then went over to flat-bottomed, cylindrical 'Lucite' cups, which could stand in a closed Petri dish. Their inner height was 8 mm, and their inner diameter 7 mm. Drugs were dissolved in distilled water, and the same volume of different drug concentrations dispensed into these cups. The cups were then dried *in vacuo* over 'Drierite', and stored in the refrigerator. Into the dried cups 0·3 ml. agar was dispensed and, after 1 h, they were inoculated.

Parallel experiments were carried out in tubes containing 10 ml. broth. Agreement was good for streptomycin. Whenever in the 10-ml. broth tubes growth was slight, in agar cups, at similar concentrations of streptomycin, only a few colonies appeared on the surface.

Unexplained inconsistencies occurred with penicillin. We suspected a loss of penicillin while drying. In order to avoid this loss, different substances were added to penicillin before drying. The following substances proved not to interfere with penicillin activity: glycerol, egg albumen and nutrient broth. Despite these additions, the differences in apparent penicillin resistance between reconstituted cups and 10-ml. broth tubes remained.

At this time it was found that, in tube dilution tests, using 10 ml. total volume of broth, inoculum size influences the results in penicillin-resistant strains[4]. In order to test whether size of inoculum is the only cause of the discrepancies, the dried penicillin was reconstituted not in agar but in 0·2 ml. nutrient broth, which already contained the inoculum. It was confirmed that inoculum size also influences the results in 0·2 ml. total volume of broth. However, results were not the same when tests were carried out in different broth volumina with the same size of inoculum.

Tests were carried out in which inoculum size (10^3, 10^4, 10^5 and 10^6) as well as total broth volume (0·2, 1, 2·5, 5 and 10 ml.) were varied. The following resistant strains were tested for penicillin sensitivity: *Staphylococcus*

Table 1. Mean Inhibition Doses of Penicillin per ml. for Different Inoculum Sizes and for Different Total Volumes of Broth

	Strain 81			
Total volume of broth (ml.)	Total inoculum 10^3	10^4	10^5	10^6
0·2	1·13	3·75	100	1,250
1·0	1·33	2·17	68	1,166
2·5	1·90	3·07	21	300
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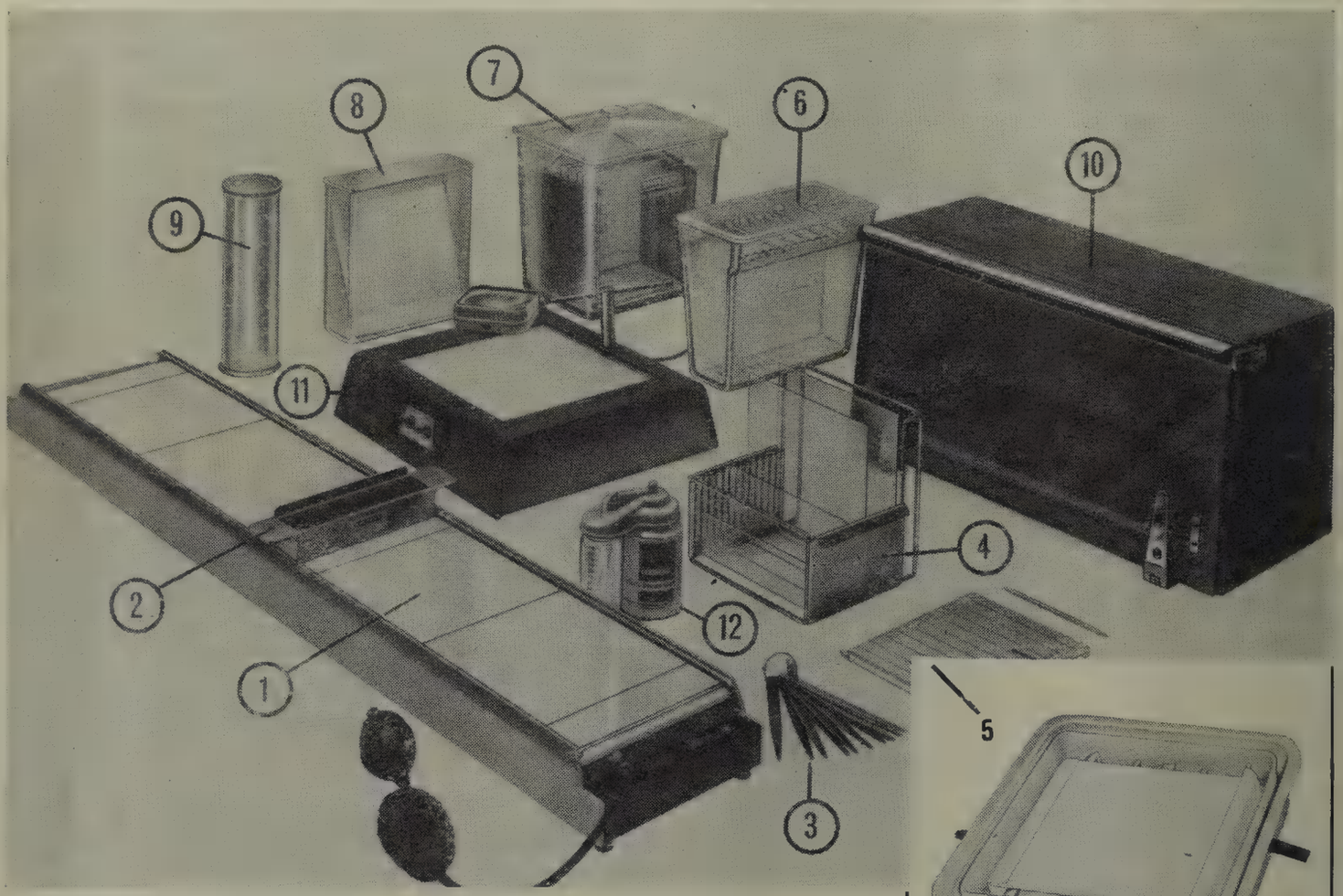

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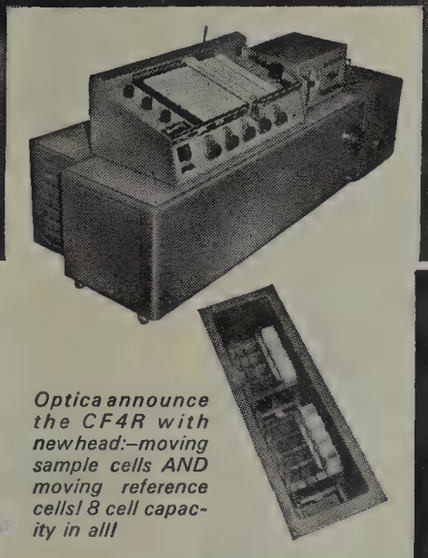

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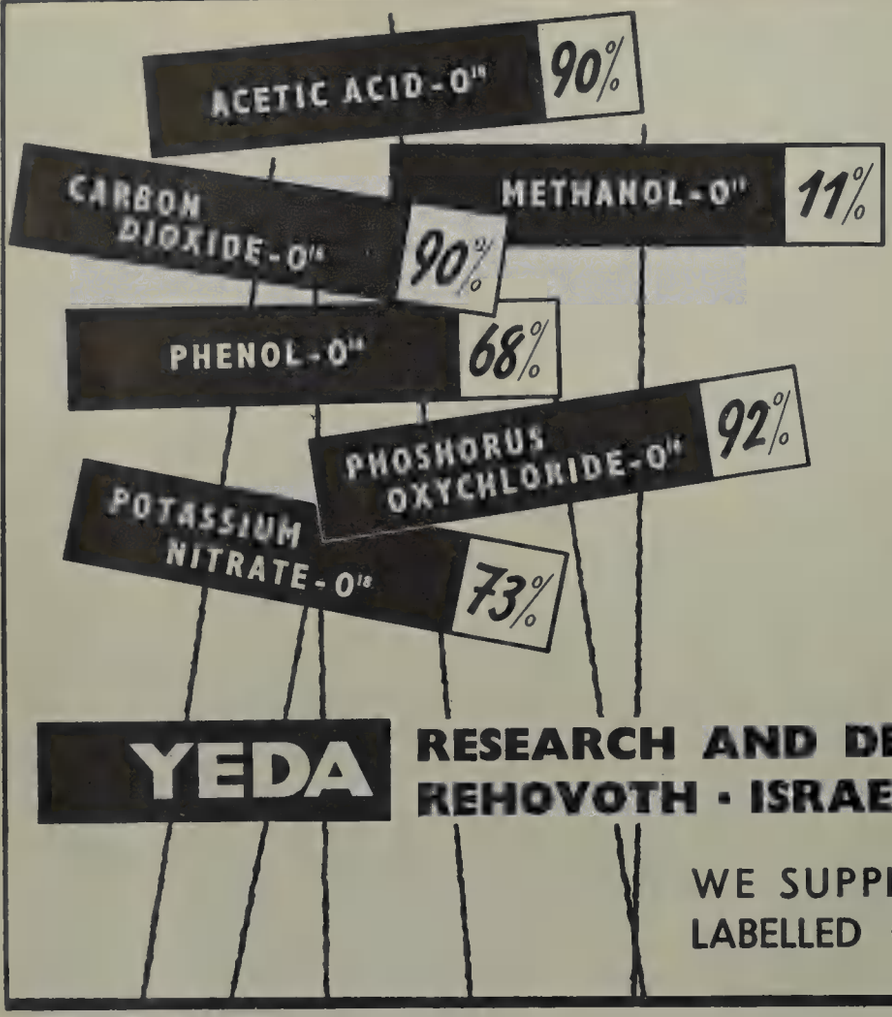

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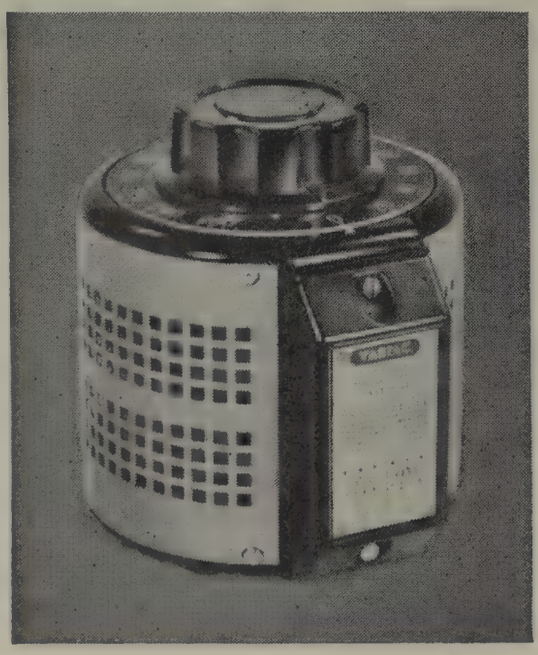

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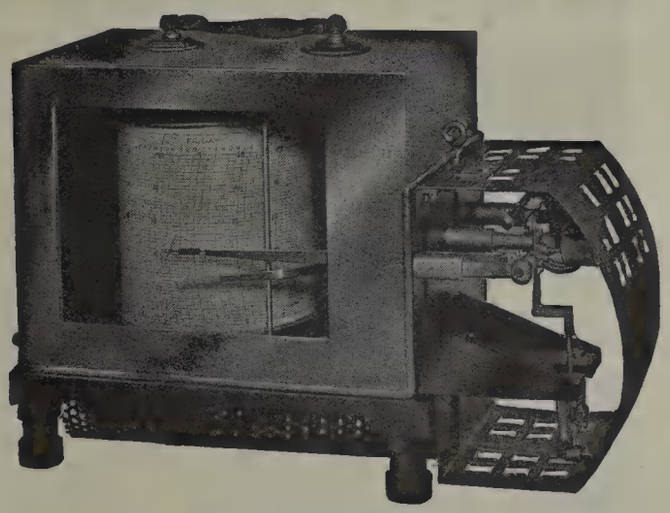

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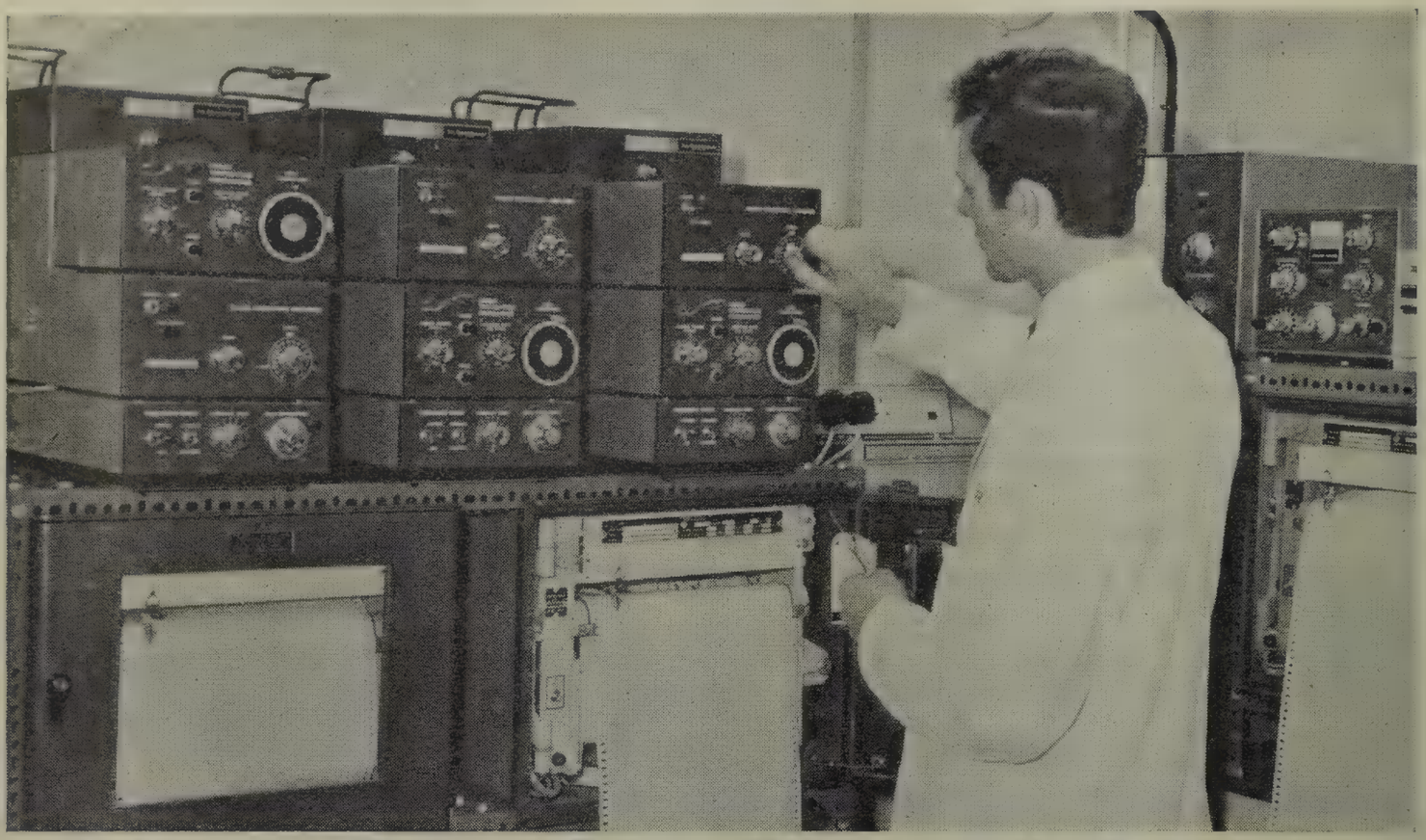

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Table 2. THE THREE RECOMMENDED DOSES PER ML. FOR PENICILLIN (*A*), (*B*), (*C*), STREPTOMYCIN (*E*), (*F*), (*G*), AND COMBINATIONS THEREOF (*J*), (*K*), (*L*) Inhibiting dose of penicillin (*D*) and streptomycin (*H*). Total inoculum: 10^6. Total volume of broth: 0·2 ml. Plus sign indicates growth; minus sign absence of growth. Penicillin in units, streptomycin in μg

Strain	Penicillin				Streptomycin				Penicillin + streptomycin		
	(*A*) 1	(*B*) 3·16	(*C*) 10	(*D*)	(*E*) 2	(*F*) 6·32	(*G*) 20	(*H*)	(*J*) 1+2	(*K*) 3·16+6·32	(*L*) 10+20
80	+	+	+	113	+	+	+	913	+	+	+
81	+	+	+	108	+	+	−	8·1	+	−	−
MG	+	+	+	47	+	+	+	175	+	+	−
45162	+	+	+	100	+	+	+	750	+	+	+
46422	+	+	+	23	+	+	+	6,125	+	+	−
65126	+	+	+	100	+	+	+	542	+	+	+

aureus 80, 81, *MG*: and three 'Oxford' strains: 45162, 46422 and 65126. As all these strains gave similar results, 81 is chosen to represent the group. Results are summarized in Table 1; figures listed are averages of at least five experiments.

From Table 1 it can be seen that the volume of broth does not play a relevant part with small (10^3 and 10^4) inocula; with larger (10^5 and 10^6) inocula, results become dependent on total volume of broth. By choosing an inoculum of 10^5 micro-organisms for a total volume of 0·2-ml. broth, the results, in all instances tested, are rendered comparable to those obtained in 10-ml. broth in the tube dilution method.

In contrast to the foregoing, penicillin-sensitive strains tested (*Staphylococcus epidermidis*, *aureus*, 52*A*, 79, and three 'Oxford' strains: 46407, 46426 and 46479) show little dependence on broth volume.

The single 'Lucite' cups were now replaced by a plastic slab, which fits into a Petri dish. Twenty-five cavities were drilled into the slab. Eight radii of three cavities each radiate from a central cavity, which serves as control (Figs. 1*a* and *b*).

Into these cavities 0·05-ml. drug solutions were dried *in vacuo* over 'Drierite'. Different drugs, in different concentrations, singly or in combination, with or without glycerol, were dried into the cavities. Glycerol, in a concentration of 0·5 per cent of the fluid to be dried, facilitates reconstitution, and imparts to the dried drugs in the cavities a glossy sheen.

The dried slabs were tested immediately after drying, and after varying storage times; after two months' storage the same results were obtained.

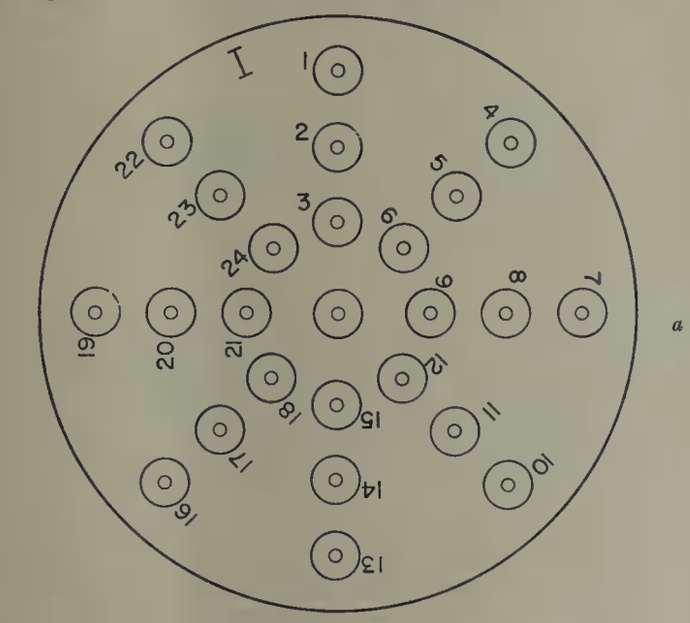

Fig. 1. *a*, Plan of slab. Twenty-four cavities are arranged in 8 radii. Each radius is formed by three cavities. The cavity in the centre accommodates the growth control. The small circles within each cavity are filled in, where growth occurs. *b*, Slab in Petri dish, cut through cavity 7 to cavity 19. Growth settles in the tip of the cavity

Slabs with dried drugs were subjected to shaking for 2 h in an inverted position; no loss in potency was observed.

For practical purposes, only three concentrations of a drug, or combinations of drugs, are needed to arrive at a valid estimate. The three concentrations are accommodated in the three cavities of a radius. One cavity contains the maximum attainable concentration of the drug in the blood; penicillin, for example, 10 units/ml. The third cavity contains one-tenth of this concentration; penicillin, for example, 1 unit/ml. The intermediate cavity of the radius contains the geometric mean, namely: $\sqrt{10 \times 1} = 3{\cdot}16$ units/ml.

An organism which grows in the presence of the highest concentration of the drug is considered resistant. An organism which grows in the presence of the intermediate concentration is considered moderately sensitive. An organism which grows in the presence of the lowest concentration only is considered sensitive. If no growth occurs at any concentration the organism is considered highly sensitive.

For combinations of drugs also, the highest concentrations are the maximum levels attainable in blood; for example, penicillin 10 units/ml. plus streptomycin 20 μg/ml. The lowest concentrations of drugs are one-tenth of the highest; penicillin 1 unit/ml. plus streptomycin 2 μg/ml. The intermediate concentrations are the geometric means: penicillin 3·16 units/ml. plus streptomycin 6·32 μg/ml. The interpretation of results is the same as for single drugs.

Table 2 illustrates results obtained from several strains.

From Table 2 it can be seen that *Staphylococcus* type 81 is penicillin-resistant (inhibitive dose 108 units/ml.), and moderately sensitive to streptomycin (inhibitive dose 8·1 μg/ml.). This organism, however, is sensitive to a combination of penicillin and streptomycin (inhibitive doses: penicillin 2 units/ml. plus streptomycin 4 μg/ml.). *Staphylococcus* 46422 is penicillin-resistant (inhibitive dose 22·5 units/ml.), streptomycin highly resistant (6,125 μg/ml.), and is moderately sensitive to the combination of both (inhibitive doses: penicillin 10 units/ml. plus streptomycin 20 μg/ml.).

On the other hand, *Staphylococcus* 65126, which is penicillin-resistant (inhibitive dose 100 units/ml.), and streptomycin-resistant (inhibitive dose 542 μg/ml.), remains resistant to the combination of both drugs.

In the proposed method all one has to do with the prepared slab is to adjust an 18-h-old culture of micro-organisms in broth so as to contain 5×10^5 bacteria/ml., and to dispense 0·2 ml. into each cavity, starting with the cavity in the centre, the control. Results are clearly read with the naked eye; even the slightest growth can be detected. A sediment mostly forms in the tip of the cavity. Results are comparable with those from the tube dilution method in 10 ml. An estimate of combined drug activity is obtained.

We thank Dr. R. L. Vollum, director, Bacteriology Laboratory, Radcliffe Infirmary, Oxford, for kindly sending us the strains.

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Sargassum TANNIN, AN ANTIBIOTIC WHICH RETARDS FOULING

By Prof. JOHN McN. SIEBURTH and Prof. JOHN T. CONOVER
Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island

THE pelagic gulfweeds, *Sargassum natans* and *S. fluitans*, which belong to the Phaeophyta, have branch tips which are virtually free of an epibiota when they are at the centre of their distribution in the Sargasso Sea gyre. These tissues possess antibacterial activity[1]. During filter-pad antibacterial assays the colour of active young tissues turned from yellow–green to black on incubation, while older and inactive preparations remained brownish in colour. This observation brought to mind the black pigment phycophaein formed by the oxidation of phaeophyte tannins[2] and the use of *Sargassum ringgoldianum* extracts in the tanning industry[3]. Extracts of actively growing branch tips were astringent, precipitated gelatin, and blackened ferric chloride solutions. These properties are characteristic of tannins, further suggesting that tannins were present in appreciable quantities in these tissues. Further observations were made during R.V. *Trident* cruise 10 (September 7–20, 1963) to the Sargasso Sea.

From samples of fouled weed a sensitive *Pseudomonas* strain was isolated and used as a test organism. This organism was more sensitive and had a shorter incubation period than the *Vibrio* isolate used previously[1]. Only in material south-east of Bermuda in the Sargasso Sea gyre was there marked antibacterial activity and the presence of tannin-like substances in the branch tips. A comparison was made of the antibacterial activity of tannic acid (U.S.P., 'Mallinckrodt') and that of *S. natans* extracts (Fig. 1). Inhibition by tannic acid was detectable at concentrations greater than 0·01 per cent and was linear above 0·1 per cent. The more active *S. natans* preparations showed inhibition equal to that of a 0·2 per cent tannic acid solution. Although the inhibitory tannic acid and algal extracts were acidic, a hydrochloric acid solution diluted to *p*H 3·85 failed to inhibit the test organism. Inhibition by tannic acid and the phaeophyte tannin may be due to their protein precipitating activity.

Since tannins by definition precipitate protein, an attempt was made to observe the effect of adding extracts of young branch tips to the epifauna of fouled weeds. Hydroids (such as *Plumaria* and *Clytia*) were ideal test organisms as their tentacles remained active even after being poked by a glass rod or being hit by drops of sea-water. Initial observations with drops of a 1 : 4 w/v suspension in 25 ml. of sea-water 'froze' the active tentacles, which became deformed and exuded strings of precipitated protein. The results with hydroids were so marked that a series of observations with different dilutions were made on a number of members of the epifauna; the results are summarized in Table 1. Dilutions of 1 : 125 affected most of the fouling organisms, while the hydroids were sensitive to dilutions as great as 1 : 1,000. Tannic acid solutions had a similar spectrum of activity with 0·0008 per cent solutions immobilizing 50 per cent of the hydroids. The tissue content of the 'tannins' appears to be sufficiently high to retard fouling.

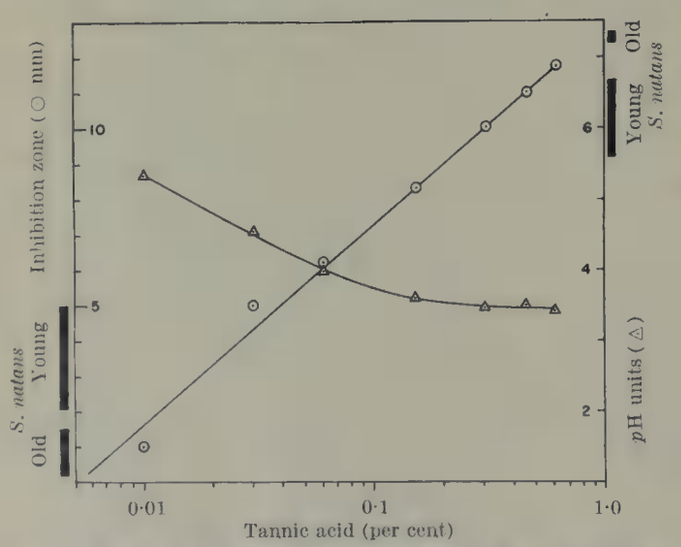

Fig. 1. Comparison of antibacterial activity and *p*H of tannic acid solutions with those obtained from young and old tissue suspensions (1 : 4 w/v) of *Sargassum natans*. A hydrochloric acid control of *p*H 3·85 did not inhibit the pseudomonad test organism

Table 1. Immobilization of Epifauna on Older Epibiota-enriched Gulf Weeds by Sea-water Extracts of Young Epibiota-free Branch Tips of *Sargassum natans*

Test organisms*	Dilution of *S. natans* slurry in sea-water 1 : 125	1 : 250	1 : 500	1 : 1,000	1 : 2,000
Hydroids[a]					
Clytia noliformis	+[b]	+	+	+	–
Other spp.	+	+	+	+	–
Bacteria[c]	+	±[d]	±[d]	–	–
Worms					
Planaried	+	–	–	–	–
Round (nematode)	+	–	–	–	–
Nereid	–[e]	–	–	–	–
Round (others)	–	–	–	–	–
Spirorbis	–	–	–	–	–
Pycnogonids	+	–	–	–	–
Copepods	+	–	–	–	–

* Observations on a number of active members of the community.
[a] Immobilization accompanied by loss of cell structure and precipitation.
[b] Complete loss of motility.
[c] An enrichment culture predominantly of *Vibrio* spp.
[d] Temporary inactivity only.
[e] Violent motions.

Several attempts were made to concentrate and purify the 'tannins'. Only young branch tips free of fouling were suitable. Extraction was made difficult by the marked decrease in antibacterial activity following collection. Even when the tissue was preserved by freezing in packed plastic bags the activity was minimal after two to three weeks. The most successful extraction was conducted aboard ship on fresh material. Young branch tips triturated in a Waring blender with sufficient distilled water to make a slurry were extracted at 50° C for 15 min, and coarse-filtered through a clean towel to yield an opalescent suspension. Most of the chlorophyll was removed by repeated ether extraction. A red-brown fraction was brought into an iso-amyl alcohol phase with sodium sulphate. The iso-amyl alcohol fraction was evaporated to dryness in a rotary evaporator, and the residue was extracted with acetone. The acetone-soluble fraction was also evaporated to dryness, and the residue was taken up in a minimal amount of water to yield a deep amber-coloured liquid. This procedure extracted half the antibacterial activity as shown by assays while reducing the volume a hundred-fold. The dried extract had the solubility characteristics of *Sargassum* tannin[2], darkened a ferric chloride solution, precipitated gelatine, and was highly astringent. The insolubility of the active material in ethyl ether separates this material from the ether-extractable antibacterial, sarganin, reported for *S. natans*[4].

The presence of tannic substances in brown algae is associated with refractile, acidic vesicles called physodes, fucosan vesicles or metachromatin granules[2,5]. The excretion of phenolic substances from the physodes of living algae has been recently reported[6]. Accumulation of these tannins was greatest in the distal tips of the thalli. Antibacterial activity in extracts of epibiont-free branch tips of *S. natans* from the Sargasso Sea was observed in early October 1962 and September 1963 (R.V. *Trident* cruises 1 and 10), as well as in week-old frozen material brought back from other summer and autumn cruises. In contrast, observations made in January 1964 (R.V. *Trident* cruise 14) indicated that even the branch tips were fouled by

hydroids and that antibacterial activity was not detectable. Both dried and preserved specimens were examined critically for physodes, which occur as yellow-refractive spherules 1μ in diameter. The active distal tissue of algae collected during cruise 10 possessed 40–68 physodes per cell in contrast to 2–12 physodes from similar but inactive tissues from cruise 14. The presence of physodes appears to be coupled with antifouling in brown algae. This is further substantiated by reports on the absence of physodes in the cells of host brown algae adjacent to the invading filaments of parasitic epiphytes[7]. The antibiotic activity of marine algae in temperate zones has been shown to vary seasonally, peaks in activity occurring during active growth and being dependent on the boreal or tropical nature of the plant[8]. A drop of 3° C in the temperature of surface waters between cruises 10 and 14 was apparently sufficient to alter the physiology and antifouling activities of these plants. These observations on the seasonal change in the number of physodes per cell, in addition to those on tannin activity and antifouling in branch tips, further indicate the excretory function of these vesicles.

The recent work of Craigie and McLachlan[6] has shown that a number of phaeophycean algae produce yellow, ultra-violet-absorbing materials which appear to be flavenols or catechin-type tannins. These substances were found to be toxic to unicellular algae at concentrations of 0·025–0·15 per cent. Concentrations of natural tannins similar to those inhibitory for our pseudomonad were also inhibitory for cellulolytic but not saprophytic bacteria[9]. The phaeophyte tannins should be included among the known algal antimicrobials[10].

These observations indicated that tissues of algae containing tannins equal in activity to 0·1–0·8 per cent tannic acid (dry weight) retard fouling in *S. natans* and that unprotected tissues such as the tentacles of hydroids are sensitive to tannic acid solutions as dilute as 0·0008 per cent. Since fouling is one of the outstanding economic problems facing marine biology, an attempt was made to see if tannic acid *in vitro* would also retard fouling. Tannic acid (U.S.P., 'Mallinckrodt') dissolved in acetone at a 15 per cent (w/v) concentration. This solution was added to varnish and lacquers in equal volumes. Control preparations were diluted with acetone alone. Similar preparations were made with paints using the cheaper grade of 'fluffy' tannic acid (N.F., 'Mallinckrodt'), but these were prone to peeling. Test panels were immersed both in Narragansett Bay, Rhode Island, and in Biscayne Bay, Florida. Although the tannic acid preparations were inferior to commercial antifouling paints, they did inhibit fouling by both barnacles and algae under both temperate and tropical conditions.

The antifouling properties of tannins were claimed as early as 1881 and paints containing tanner's bark, poke-root bark, pine bark, kelp and mangrove tree extracts have been granted U.S. Letters Patents as antifouling compositions of matter[11]. Although tannins may be effective inhibitors to bacterial, algal and animal fouling organisms in young and actively growing tissues of at least the brown algae, their economic application is undoubtedly limited.

I thank colleagues and students, S. P. Meyers, Institute of Marine Science, Miami, Florida, and James H. Ware, International Minerals and Chemical Corporation, Skokie, Illinois, for their assistance. This work was supported in part by a grant from the U.S. Public Health Service (*WP*–00100–02) and a contract with the U.S. Office of Naval Research (*Nonr* 396–08).

Note added in proof. Subsequent investigationshave shown that tannins from *Ralfsia verrucosa* affect the survival of planktonic animals in tide-pools (*Proc. Fifth Intern. Seaweed Symp.*, Halifax, August 1965).

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COMPARISON OF THE CHORIOALLANTOIC MEMBRANE AND SPLENOMEGALY SYSTEMS OF GRAFT-VERSUS-HOST ASSAY IN THE CHICK EMBRYO

By L. W. COPPLESON* and Dr. DONALD MICHIE†

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THE chick embryo can be used as the arena of graft-versus-host reactions in two ways that are convenient to measure. Intravenous injection of immunologically competent cells results in an enlarged spleen which may be weighed[1]. If a suspension of adult chick leucocytes is placed on the dropped chorioallantoic membrane (CAM) of a developing chick embryo, small white foci appear which may be counted[2–4]. This article describes the biometrical properties of the two systems as assays of immunological activity, and compares their efficiencies. The materials and methods used have been described elsewhere[5,6].

Assay systems

The essential feature of an assay is the expression of the concentration of some biologically active principle in a test preparation in terms of a standard preparation. Estimates obtained therefore take the form of a relative potency. In illustration, we present a single assay more fully, in which the CAM reaction was used.

The material to be assayed consisted of frozen adult chick buffy coat cells[6]. The cell concentration of the test suspension was 4/3 times greater than that of the standard (unfrozen) suspension in order that the same part of the dose-response curve should be used as nearly as possible The test cells, suspended in a solution containing 15 per cent dimethylsulphoxide and 30 per cent chick plasma, were frozen to −196° C and kept there for 0·5 h. The standard and test were each applied at three dose-levels, 1 in 1, 1 in 3 and 1 in 9; making 6 batches (that is, a 6-point assay[7]). A seventh batch of saline-treated eggs was included as a check for non-specific lesions, which occurred sufficiently rarely to be ignored. Six eggs were assigned to each batch.

The estimation of relative potency (relative immunological reactivity per cell) is illustrated in Fig. 1, using data from the first week of a series of seven replicates.

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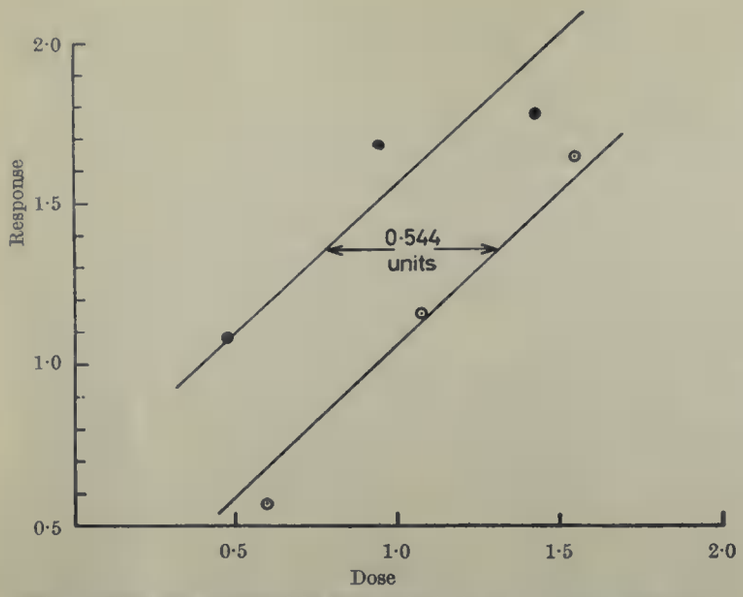

Fig. 1. Illustrative assay of the immunological potency of a suspension of adult chick leucocytes using the CAM reaction. Serial three-fold dilutions were used: all doses are expressed as log cell concentration. The response is expressed as log (No. of foci + ½). ●, Standard; ○, test

The two parallel lines are the calculated lines of best fit, and the relative potency can be read off by taking the antilog of 0·544 which is 3·5 approximately, that is, the standard preparation has 3·5 times the potency of the test. Actually it is conventional to express the test in terms of the standard, so that we would say that the relative potency is 1·0/3·5, that is, 0·29.

Parameters of the CAM system

Choice of interval. Inoculations were made on day 12 to conform with the practice of the Burnet group[2,3,8]. Day 13 was tried but produced no appreciable difference from day 12. The membranes were collected 4 days after inoculation. Examination of day 5 and isolated trials of longer periods revealed such problems in the identification and counting of individual foci that the counts were considered less reliable than those obtained with a 4-day interval.

Numerical expression of response. In the CAM response, as has been shown elsewhere[5], the scatter around the mean is grossly dependent on the mean itself, and hence on dose-level. Logarithmic transformation was found adequately to correct this feature. Since the counts occasionally contained zero values and the logarithm of zero is an infinite negative quantity, the transformation used was $\log_{10}$ (No. of foci + ½) rather than $\log_{10}$ (No. of foci).

Parameters of the splenomegaly system

Choice of interval. Day 13 was chosen for intravenous injections as being technically extremely convenient and also because Solomon and Tucker's results[9] indicate that maximum reactivity is developed at about this stage. Intervals of 3, 4 and 5 days were tested in two batches of Stirling eggs and one of inbred eggs (*C*-Line, Poultry Research Centre, Edinburgh). Using as the criterion of efficiency the ratio of the observed spleen enlargement to its standard deviation, the indicated order of preference in each case was 4 days, 5 days, 3 days. Four days was chosen as the interval for all subsequent work.

Numerical expression of response. The classical index of splenomegaly used by Simonsen was the ratio of the spleen weight in treated animals (mice) to that in littermate controls. In more recent work it has been customary to work with the logarithm of this ratio. In the chick the need for controlling inter-litter variation does not arise, so that the single measure $\log_{10}$ (spleen weight) has been used throughout. Before standardizing on this measure one other possibility was investigated, namely, $\log_{10}$ (spleen weight of treated embryos—mean spleen weight of untreated embryos). The expression inside the brackets measures the extra tissue present in the organ as a consequence of treatment. This transformation, while resulting in an improved slope, caused a deterioration in variance sufficient to counterbalance the gain.

Comparison of the CAM and splenomegaly systems

It was first pointed out by Gaddum[10] that an objective measure of the efficiency of an assay system can be calculated from the dose-response slope, b, and the within-batch standard deviation, s. The quantity s/b is usually regarded as a convenient inverse measure of efficiency, having the property that its square, s^2/b^2, is directly proportional to the number of animals which need to be used to attain a given precision in the estimate of relative potency[11]. In an examination of 45 parallel-line assays for various drugs, Bliss and Cattell[12] found values of s/b ranging from 0·03 to 0·50.

Table 1. Relative Efficiency of CAM and Spleen Assays in Stirling (*S*) and *RIR* × *LS* Material

Stirling (*S*)		*RIR* × *LS*	
CAM assays*	Spleen assays*	CAM assays†	Spleen assays
0·56 (17)	0·37 (3)	0·30 (5)	0·47 (4)

Figures represent the mean of s/b which is an inverse measure of the efficiency of the assay. Figures in parentheses denote the number of replicate assays performed.

* Includes data from ref. 6.

† From ref. 5.

In Table 1 we have set out some measures of efficiency obtained from 29 separate assays. It appears that in the Stirling embryos the splenomegaly system is more efficient whereas in the *RIR* × *LS* cross-breds the CAM system is more efficient. This interaction between genotype and test system is a real effect. If we compare the *S* − (*RIR* × *LS*) difference on the CAM test with that obtained on the splenomegaly test, the difference is significant at the 1 per cent level.

On the grounds of efficiency alone there is thus little to choose between the two methods of assay, the most important consideration being to fit the choice of recipient material to the assay system. Given suitable strain combinations, the choice between the two systems becomes primarily a matter of technical convenience. In the spleen assay it takes longer to administer the doses, but the response is less tedious and more objective to read. Therefore, for simple relative potency estimations the splenomegaly system is to be preferred. In the CAM system, on the other hand, inoculations are quick and easy, but the response is laborious and difficult to read. Consequently, the circumstances under which the CAM system is likely to be preferred are those in which it is desired to take advantage of the 'one focus, one competent cell' relation postulated by Burnet and supported by our own investigations[5].

We thank Prof. M. F. A. Woodruff, Department of Surgical Science, University of Edinburgh, and Prof. M. Simonsen, Blond Laboratories, Queen Victoria Hospital, East Grinstead, for encouragement and provision of laboratory facilities.

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FINE STRUCTURE OF WOOD

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ALTHOUGH the advent of electron microscopy, and in particular the replica and shadowing techniques, has revealed the microfibrillar orientation in the lamellae of the primary wall (P) and the secondary wall layers (S_1, S_2 and S_3), it has contributed little to our knowledge of cell wall capillaries or of intermicrofibrillar spaces. For general physiological reasons it is believed that cell wall capillaries exist; but until recently this belief was based on indirect evidence such as the weight/weight uptake of liquids by wood, or the encrustation of tracheid fragments with iodine[1] and the X-ray scattering of untreated and gold impregnated wood before and after delignification[2]. More recently it was shown that electron diffraction diagrams of *Pseudotsuga menziesii* treated with inorganic solutions were consistent with cell wall penetration; the walls stained with dithio-oxamide throughout (but patchily), the middle lamella being heavily stained[3–6]. It was noted that the resulting complex was dichroic and similar dichroism has been found by other workers using different inorganic solutions in other softwood species[7,8]. Such findings have been taken to indicate[3–6] that the complexes are oriented parallel to the microfibrils of the cellulose fraction in these walls.

It has been found by Wardrop and Davies[8] that when *Pinus radiata* is stained with congo red the layers S_1 and S_3 stain strongly, whereas the S_2 stained less strongly with the middle lamella barely staining. The authors suggested that if it were accepted that the degree of staining was indicative of the presence of capillaries of a particular minimum size, the observation would indicate that the capillaries in the true middle lamella were smaller than those of other regions; furthermore, the number of capillaries into which the dye could penetrate was greater in the layers S_1 and S_3 than in the layer S_2. Optical microscopy and low-magnification electron microscopy have shown that copper-treated *P. radiata* contains tracheids in which the copper is in or is external to the layer S_1 (ref. 9); no fine structure was revealed in this work. Mention must be made of the pioneering work of Stamm[10], whose investigations on flow demonstrated the existence of pores in the cell walls of wood cells. Similar work[11] based on electrical conductivity has recently confirmed that pores other than those in the pit membrane must traverse the cell wall completely.

Only one electron micrograph has been published[5,6]† which illustrates that a cell wall capillary may be filled or partly filled with an inorganic salt; in this micrograph, a few isodiametric deposits of a copper–chrome–arsenic wood-preservative may be seen in the cell wall of a tracheid of *P. menziesii*.

Other plant fibres are known to contain intermicrofibrillar spaces; but again it cannot be said that these are true capillaries because of the techniques adopted to reveal them. Wardrop[2] has shown that in sections of fragments of delignified flax fibres treated with gold chloride solutions it was possible to see an irregular deposition of the salt. It was assumed that surface crystallization had commenced and that aggregates had formed in the 'grooves' between adjacent microfibrils. More recently, macerated ramie fibres treated with inorganic salts solutions have yielded excellent electron micrographs showing oriented deposition of metal in the cell wall[12].

A study of the cell wall capillaries and pit membranes in wood has now been undertaken because of its wider implications in the field of wood-preservation. It was assumed that interstices could occur between and parallel to the microfibril, and that interstices in adjacent lamella would sometimes be linked, probably randomly, so that in effect a capillary system across and along the cell walls could exist. In theory it was thought that such a capillary system would allow the passage of polar liquids (but probably not non-polar liquids) from cell lumen to middle lamella, and, if this were also permeable, to the cell wall of the adjacent cell. Cutting of ultra-thin sections parallel to the microfibrillar orientation in metal-containing wood should then reveal the capillaries as elongated electron-dense zones, with more isodiametric zones indicating interstices linking lamellae.

Sapwood samples (3 in. long. × 1 in. tang. × 1 in. rad.) from two species of the hardwood genus *Eucalyptus* were placed under vacuum to remove air and then pressure-treated at 70 lb./in.^{2} with aqueous solutions, either 10 per cent aqueous silver nitrate followed by 10 per cent aqueous hydrazine hydrate, or with a 10 per cent aqueous copper–chrome–arsenic wood-preservative ('Tanalith *C*'). After air drying, thick sections (30μ) were cut at a small angle to the cell axis and portions of these embedded in meth-acrylate, 'Araldite' or 'Epon'. Thin sections (500 Å) were cut and examined in the electron microscope. Fig. 1 illustrates silver deposition in the capillaries in the S_2 and S_3 layers of a fibre tracheid from *E. obliqua* sapwood sections cut approximately tangentially to the S_2 microfibrillar direction. From this and similar electron micrographs it has been found that the average diameter of the deposits, and therefore approximately of the capillaries, is about 300–350 Å, no corrections being made for deformation due to crystal expansion during its growth. This same electron micrograph also shows limited evidence of the orientation in the S_3 layer.

The appearance of the tracheid wall of *E. maculata* sapwood impregnated with 'Tanalith *C*' (Fig. 2) and silver (Fig. 3) is somewhat different. Here, capillaries appear much smaller (approx. 100 Å) though, again, they can be seen to be oriented in relation to the microfibrils, but only certain areas are readily penetrated by the aqueous solutions. In Figs. 1, 2 and 3 small isodiametric electron-dense zones are also to be seen, indicating capillaries connecting adjacent lamellae.

Because of the importance of pits in any examination of liquid flow in wood, the structure of these has also been investigated. No evidence of large pit membrane pores has been found in *E. obliqua* and *E. maculata*; but the membranes exhibit a random pattern of interstices filled with the appropriate inorganic constituent (Fig. 4). In a few instances the pit membranes have been found to be torn (presumably during the initial sectioning) so that two thin membranes appear to be present. These findings are consistent with the belief that the membrane is composed of two primary walls without lignification and without a middle lamella.

No evidence has been found to indicate that the middle lamella is preferentially penetrated such as has been found previously[5,9] with softwoods. However, other workers[8] found that no copper was present in the middle lamella when sections of *P. radiata* were cut from copper-

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† *Note added in proof.* See also Preston in *Cellular Ultrastructure of Woody Plants*, ed. Côté (Syracuse Univ. Press, 1965).

Fig. 1

Fig. 2

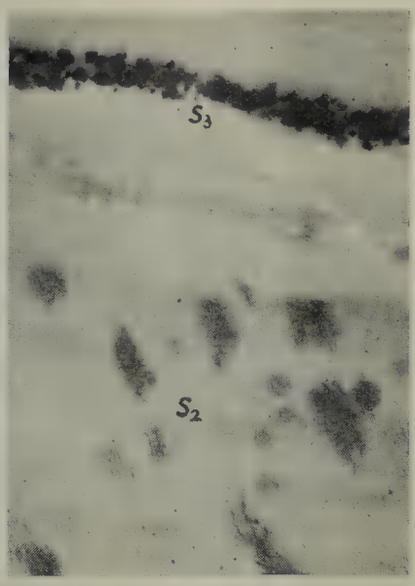

Fig. 3

Fig. 1. Electron micrograph of a fibre tracheid of *E. obliqua* containing silver in the cell wall capillaries (unshadowed longitudinal section × 23,000)

Fig. 2. Electron micrograph showing the deposition of a copper–chrome–arsenic wood-preservative in the tracheid wall capillaries of *E. maculata* (unshadowed longitudinal section, × 15,000)

Fig. 3. Electron micrograph of *E. maculata* showing silver deposited in the tracheid wall capillaries (unshadowed longitudinal section, × 23,900)

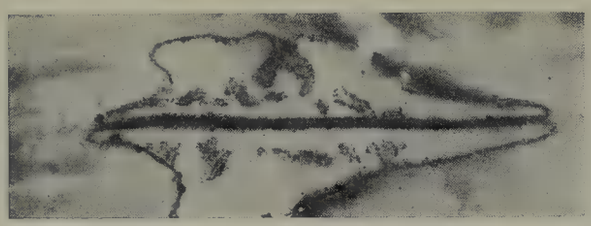

Fig. 4. Electron micrograph of *E. maculata* in which the structure of the pit membrane of a vestured pit is illustrated by the deposition of silver (unshadowed longitudinal section, × 10,860)

Fig. 5. Electron micrograph of *E. maculata* showing silver deposited in the end wall of a vertical parenchyma cell (unshadowed longitudinal section, × 5,700)

treated blocks, but could be detected if sections were soaked in copper solutions.

Electron microscopy carried out in the work recorded here has revealed that after pressure impregnation with inorganic solutions the cell lumina are lined with inorganic salts, and it is usual to find that the S_3 layer has been penetrated fairly frequently. An examination of the figures will show that the S_3 layer is quite an open network, with large capillaries. The rest of the wall is much less frequently penetrated and this could be due, for example, to hydrophobic cytoplasmic debris blocking the capillaries in the S_3 layer. The rather unusual silver deposition in a parenchyma cell of *E. maculata* which is shown in Fig. 5 provides an interesting illustration of relative penetrabilities of the various cell wall layers. It will be seen that, once the initial barrier is penetrated, movement through capillaries occurs and liquids may cross the middle lamella. Significantly, and in general agreement with the findings based on indirect staining investigations[8], many of the capillaries in the middle lamella are smaller than those in other regions of the cell wall.

This work has illustrated that the cell walls in eucalyptus contain fine capillaries up to 450 Å in diameter, that these may be traversed by aqueous solutions which gain access from the cell lumen, that the middle lamella offers no resistance to flow and that direct access to the middle lamella from the pit chamber (Fig. 4) is not possible. Though the pit membrane contains no large pores, aqueous solutions can pass through it.

When this work is more fully reported elsewhere and the practical implications for the wood-preserving industry discussed, photomicrographic evidence will be presented to illustrate that the pits in eucalypts form the major pathway by which aqueous solutions travel from cell to cell, with the cell wall capillaries serving mainly to distribute the solution to the walls.

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A NEW CHEMICAL ASPECT OF PENICILLIN ALLERGY: THE DIRECT REACTION OF PENICILLIN WITH ε-AMINO-GROUPS

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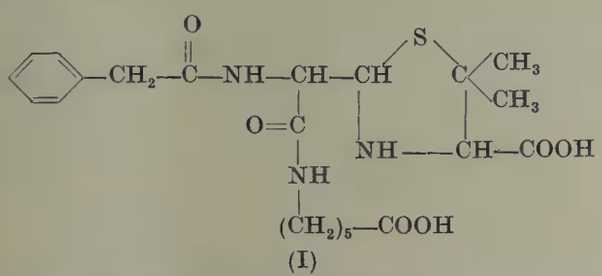
IT seems well established that the penicilloyl group bound to carrier protein structures *in vivo* is a main antigenic determinant in penicillin allergy[1–3]. On the other hand, the chemical basis for the formation of these penicilloyl protein conjugates has not yet been satisfactorily elucidated. Earlier views on the formation of the penicilloyl haptenic structure have held that a highly reactive degradation product of penicillin, penicillenic acid, might be the only form in which penicillin reacts irreversibly with proteins. Penicillenic acid is, in fact, spontaneously formed from penicillin in neutral solution and is able to react with free amino-groups, thereby forming a penicilloyl-amide[4]. The possibility that penicillins could react directly with ε-amino-groups by an opening of the β-lactam ring has been neglected mainly because an experimental demonstration of the reaction between penicillins and such amino-groups on proteins, peptides or low molecular weight amines in neutral aqueous solution was lacking[5].

Two facts have been known for some time to throw some doubt on the concept of penicillenic acid being a main intermediate in the formation of the penicilloyl haptenic structure: (*a*) There is no correlation between the *in vitro* rate of formation of penicillenic acid from various penicillins bearing different acyl side-chains and the immunogenicity of these penicillins; (*b*) 6-aminopenicillanic acid (6-APA), being structurally incapable of rearrangement to a penicillenic acid intermediate, was shown to be a strong inducer of the allergic state in rabbits[6].

In experiments initiated by these considerations, the incubation of penicillins with amines in neutral aqueous solution was examined. It was established that penicillins do react with amines under such conditions to yield penicilloyl-amides. In this article we report in a preliminary form some of our results together with findings obtained with dinitrophenyl-6-aminopenicillanic acid, demonstrating that the direct reaction of the β-lactam of penicillins with amines is an important general route to the penicilloyl-amide antigenic determinant. (A full account of this work will be published in *Helv. Chim. Acta.*)



(I)

Benzylpenicillin (0·5 M) was incubated at 37° with ε-aminocaproic acid (molar ratio, 1 : 4·25) in 0·5 M phosphate buffer *p*H 7·4, and also in 0·1 M sodium bicarbonate at *p*H 7·4. Additions of small amounts of sodium hydroxide kept the *p*H at the desired value. After one day a viscous gum was precipitated from the reaction mixture by acidification. From the precipitate, crystalline benzylpenicilloyl-ε-aminocaproic acid (I) in the form of its dibenzylammonium salt was obtained. The products obtained in the two runs agreed in properties (m.p. 110°–113° and 111·5°–113°. Corrected capillary melting points are reported; $[\alpha]_D^{24} = +69{\cdot}7°$ and $+69{\cdot}2°$ (c = 1, water); penamaldate assay[7]: $E_m = 24{,}000$) with an authentical sample of benzylpenicilloyl-ε-aminocaproic acid dibenzylammonium salt prepared in alkaline solution. Mixed melting points were not depressed. The yields were 20–30 per cent. In addition, benzylpenicillin was similarly incubated at physiological *p*H with poly-L-lysine of mean molecular weight about 1,000, the molar ratio of penicillin to ε-amino-groups being 1 : 0·4. Passage of the incubation mixture through a 'Sephadex *G*-25' column neatly separated the polylysine material (peak I) from the residual material (peaks II and III) (Fig. 1). Penicilloyl groups attached to the polylysine were measured by a modified penamaldate assay[7]. In accordance with the behaviour expected for a benzylpenicilloyl polylysine[7], the material from the column (peak I) specifically precipitated antisera from rabbits which had been immunized with benzylpenicilloyl-bovine γ-globulin.

Other penicillins were incubated for 4 h at 37° with polylysine in 0·05 M phosphate buffer *p*H 7·4 at a concentration of 75 mM, the molar ratio of penicillin to ε-amino-groups being 1 : 1. Polylysine material was isolated from the incubation mixture through 'Sephadex *G*-25' gel-filtration and the amount of penicilloyl-amide on the polylysine was established by penamaldate analysis. That no other material developing under the conditions of the incubation and detectable by penamaldate assay, was carried with the polylysine peak, was verified in each case by a run in which polylysine was added only at the end of the incubation period. The polylysine peaks

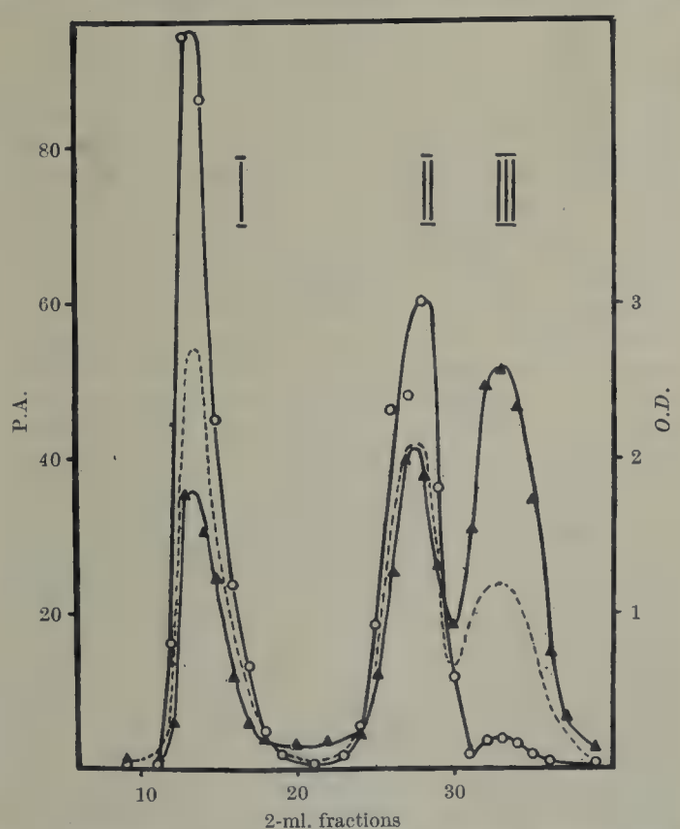


Fig. 1. Gel-filtration of 1 ml. of a solution of benzylpenicillin (250 mg) and polylysine (40 mg) in 4·0 ml. of McIlvaine's citrate-phosphate buffer incubated for 24 h at 37°, *p*H 7·4. The column (1·8 cm × 30 cm) containing 'Sephadex *G*-25' was equilibrated and eluted with McIlvaine's citrate-phosphate buffer. Analysis of eluate: ○, penamaldate assay (P.A.) as increase in optical density at 282 mμ after $HgCl_2$ treatment: ▲, optical density at 257 mμ; – – – Folin-Lowry colour (ref. 8) as optical density at 750 mμ. Optical measurements were made in 1 cm cells

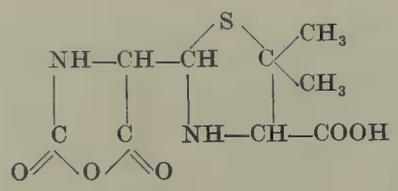
Table 1. Aminolysis of Penicillins and Degradation to Penicillenic Acid

Penicillins	Penicilloyl groups on polylysine after incubation of 300 μmoles of penicillins with excess polylysine for 4 h at *p*H 7·4, at 37° μmoles*	Degradation to penicillenic acid at 37°, from (ref. 6) Mole parts per thousand per min at *p*H 4·0	Mole parts per thousand per h at *p*H 7·4
Benzylpenicillin	19·3	2·0	0·26
Phenoxymethylpenicillin	21·1	0·06	0·008
Allylthiomethylpenicillin	22·7	0·5	0·06
Phenoxyethylpenicillin	22·6	0·08	0·01
Dimethoxyphenylpenicillin	12·3	2·5	0·29

* Calculated from the penamaldate assay with a mean molar extinction of 24,000.

obtained in these control runs did not contain significant amounts of material detectable by penamaldate analysis. The results are shown in Table 1.

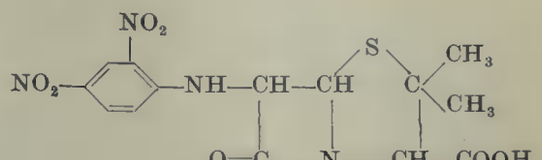
These experiments demonstrate the formation of the penicilloyl-amide bond in aqueous solutions at physiological *p*H. They make it unlikely that the route to penicilloyl-amides proceeds in general through penicillenic acid as a main intermediate, since no correlation between conversion to penicillenic acid of the various penicillins and the formation of penicilloyl polylysine is found (Table 1).



(II)

However, the use of a penicillin-like molecule unable to undergo the penicillenic acid rearrangement, but capable of acylating amines in neutral aqueous solution and of inducing the formation of specific antibodies *in vivo*, is required in order to demonstrate unambiguously that the direct acylation of amino-groups by the penicillin molecule through opening of the β-lactam ring, without involvement of a penicillenic acid type intermediate, occurs and constitutes an important route *in vivo* to the penicilloyl-amide antigenic determinant. 6-APA would seem to be a good choice in this respect. However, this compound is peculiar as it reacts easily with carbon dioxide, thereby yielding 8-hydroxypenillic acid[9–11]. The reaction probably involves a CO_2-6-APA adduct (II) (ref. 9) as an intermediate which conceivably could react with amines. Furthermore, 6-APA is capable of intermolecular acylation by reaction of the amino-group of one 6-APA molecule with the β-lactam ring of another. On repetition of this process, polymers of 6-APA are formed for which evidence has been reported[12,13]. Since the dimer penicillanyl-aminopenicillanic acid as well as the higher polymers are, in fact, penicillins, they are in principle capable of the penicillenic acid rearrangement. Therefore, the occurrence of a reaction between 6-APA and amines at neutrality could be considered as proof of a direct reaction of the β-lactam with amines only if the reaction products were identified as penicillanyl amides, or if additional information indicated that the contribution of the polymerization under the conditions used is negligible. On the other hand, the first step of the 6-APA polymerization, the *N*-acylation of a 6-APA molecule by a second one, obviously cannot proceed via a penicillenic acid intermediate and constitutes a demonstration of the direct attack of an amino-group on the β-lactam. However, the equivalence in this respect of the reacting amino-group of 6-APA and ε-amino-groups could not be taken for granted.

We have obtained preliminary evidence that 6-APA reacts with polylysine in neutral aqueous solution in a nitrogen atmosphere free of carbon dioxide to yield a conjugate which specifically precipitated a rabbit anti-benzylpenicilloyl antiserum. The reaction was followed by penamaldate assay. It seemed diminished in sodium bicarbonate solution. We have further observed that, in the absence of polylysine and carbon dioxide, a considerable increase of material showing a penamaldate assay results on incubation of 6-APA alone in neutral solution. This could be due to hydrolysis but in part also to polymerization of 6-APA.



(III)

In view of the complications connected with 6-APA work, we proceeded with dinitrophenyl-6-aminopenicillanic acid (DNP-APA) (III). This substituted 6-APA contains all the features of penicillins required for the demonstration of a direct aminolysis of the β-lactam since a penicillenic acid rearrangement cannot occur and potential side-reactions of 6-APA are avoided by the blocking of the 6-amino-group. The compound was prepared by reacting 6-APA with dinitrofluorobenzene in a mixture of acetone and sodium bicarbonate solution. The DNP-APA could be well crystallized as the free acid from ether and from acetone–water (m.p. 164°. Analysis, calc. for $C_{14}H_{14}O_7N_4S$: C, 43·98; H, 3·69; N, 14·65; found: C, 44·23; H, 3·90; N, 14·58; neutralization equivalent, calc. 382·3; found: 383).

This compound (2·5 g) was incubated with ε-aminocaproic acid (3·8 g) in 0·1 M phosphate buffer (8 ml.) at 37° for one day. The incubation solution was kept at *p*H 7·2 with the aid of a *p*H-stat, delivering 1 N sodium hydroxide to the reaction vessel. Dinitrophenyl-6-aminopenicillanyl-ε-aminocaproic acid was isolated in the form of its crystalline dibenzylammonium salt in nearly 40 per cent yield (m.p. 135°–137°. Analysis, calc. for $C_{34}H_{45}O_8N_7S$: C, 56·10; H, 6·23; N, 13·47; found: C, 55·84; H, 6·71; N, 12·93; penamaldate assay: E_m = 25,000). Manipulations were performed in subdued light, since the desired product as well as DNP-APA itself are light-sensitive in neutral solution.

The formation of dinitrophenyl-6-aminopenicillanyl-ε-aminocaproic acid from DNP-APA and ε-aminocaproic acid in 0·05 M phosphate buffer at *p*H 7·4 was compared to the formation of benzylpenicilloyl-ε-aminocaproic acid from benzylpenicillin and ε-aminocaproic acid under the same conditions. To this end, solutions (0·36 M) of DNP-APA and benzylpenicillin in 0·05 M phosphate buffer were incubated in a *p*H-stat at *p*H 7·4 at 37° alone and in the presence of 1·45 M ε-aminocaproic acid. The opening of the β-lactam ring was followed by penamaldate assay[14]. Figs. 2 and 3 show the very similar rates of

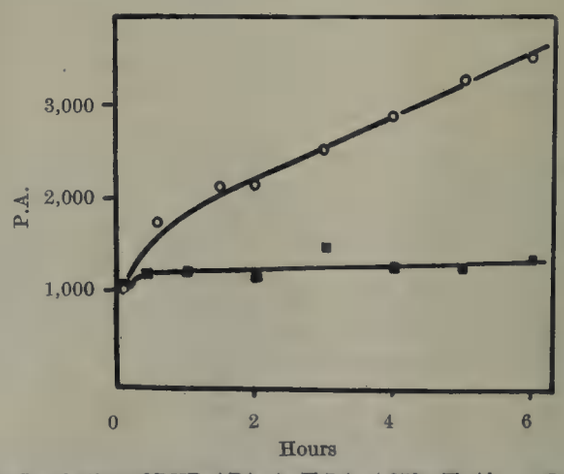


Fig. 2. Incubation of DNP–APA at *p*H 7·4, at 37°. ■, Alone; ○, in the presence of ε-aminocaproic acid. Penamaldate assay (P.A.) ▬ increase in optical density at 282 mμ after $HgCl_2$ treatment (1 = 1 cm)

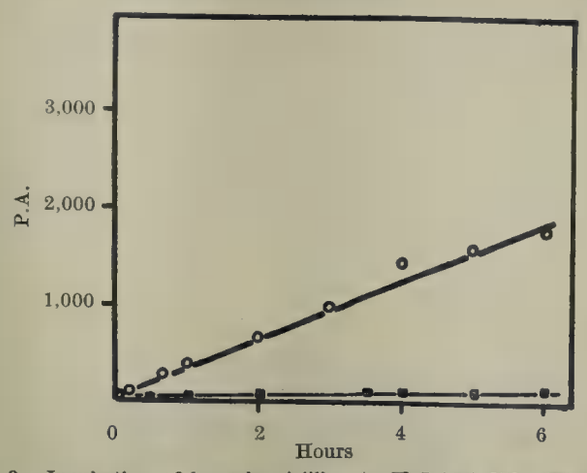


Fig. 3. Incubation of benzylpenicillin at *p*H 7·4 at 37°. ■, Alone; ○, in the presence of ε-aminocaproic acid. Penamaldate assay (P.A.) as increase in optical density at 282 mμ after $HgCl_2$ treatment (1 = 1 cm)

formation of the dinitrophenyl-6-aminopenicillanyl group and the benzylpenicilloyl group in the presence of ε-aminocaproic acid. The rate of hydrolysis of both DNP-APA and benzylpenicillin appears negligible under the conditions used. This result indicates similar rates of aminolysis for DNP-APA and benzylpenicillin and implies that the contribution of the penicillenic acid route to the aminolysis of benzylpenicillin could only be small under the *in vitro* conditions used.

The immunogenicity of DNP-APA was tested in rabbits. Animals were injected with DNP-APA and complete Freund's adjuvant (Difco) according to a procedure described previously[1]. Antibodies specific for the dinitrophenyl-6-aminopenicillanyl determinant were demonstrated in the rabbit sera by haemagglutination[1]. For the haemagglutination reaction, rabbit antisera absorbed with normal sheep erythrocytes were incubated with sheep erythrocytes previously treated with DNP-APA or benzylpenicillin in 0·1 M veronal buffer at *p*H 9·0. The haemagglutination titres of anti-DNP-APA sera with DNP-APA treated cells were comparable to those obtained with anti-benzylpenicillin rabbit sera and benzylpenicillin treated cells. The haemagglutination reaction was specifically inhibited by dinitrophenyl-6-aminopenicillanyl-ε-aminocaproic acid and by benzylpenicilloyl-ε-aminocaproic acid. The former compound was a more effective inhibitor. Only 1×10^{-4} M dinitrophenyl-6-aminopenicillanyl-ε-aminocaproic acid was required in order to achieve the same degree of haemagglutination inhibition as obtained with 6×10^{-3} M benzylpenicilloyl-ε-aminocaproic acid.

The experiments described here demonstrate the reaction of penicillins with ε-amino-groups at physiological *p*H. It is shown that the resulting penicilloylamides are formed by direct penicilloylation without necessarily involving a penicillenic acid intermediate. Even in the case of benzylpenicillin, the contribution of the penicillenic acid route appears small under certain *in vitro* conditions. DNP-APA, which has no possibility of reacting via a penicillenic acid intermediate, was shown to be immunogenic in rabbits. It is therefore suggested that the direct penicilloylation of carrier protein structures is a general route to the penicilloyl antigenic determinant *in vivo*.

While this article was being prepared, the paper by Batchelor, Dewdney and Gazzard[15] came to our notice. It is remarkable that some of the lines of thought were quite similar in the two laboratories.

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I thank Mrs. E. Stäuble for assistance.

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OSMOTROPOTAXIS IN THE HONEY-BEE

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OLFACTORY orientation in insects can follow two different paths: (*a*) Sampling of the scented area with movable antennae in temporal sequence makes it possible to perceive differences in the concentration of the scent and to make adjustments in a klinotactic orientation. This alone can be carried out with a single sensory organ on one side. (*b*) Simultaneous spatial perception of the scent gradient by both antennae: the animals succeed in orienting themselves tropotactically to the scent source. That two sense organs lie symmetrically on the body must be assumed (theoretically receptor can replace the word sense organ[1]).

Many authors believe that osmotropotactic orientation can be shown in one-antenna animals. One-antenna animals move in circles toward the antenna side in a scent current, in a different scent area, and also in a scent gradient, *Geotrupes silvaticus* Panz. and *Geotrupes vernalis* L.[2], *Calliphora erythrocephala*[3], *Leptinotarsa decemlineata* Say[4], meal-worm beetle[5], *Drosophila melanogaster*[6,9], *Habrobracon juglandis* Ashmead[7], *Ips curvidens*[8], and silkworm[10].

On the other hand, experiments have been performed with one-antenna animals as proof of a klinotactic olfactory orientation. The animals with one antenna still show a fully developed olfactory orientation; sometimes they show only a slight deviation towards the antenna side. Attaining the goal results from klinotaxis, and for this one antenna suffices for *Rhodnius prolixus*[11], *Nemeritis canescens*[12] and *Geotrupes silvaticus* Panz[13].

Other authors discuss olfactory orientation as quite a different mechanism. The scent acts only as a stimulant. Actual finding of the objective is only possible when an air current gives the animal the chance to fly rheotactically against the scent gradient[6,14–17].

For the first time we have succeeded in showing osmotropotactic orientation in a scent area for bees[18,19]. The way it works, its quantitative effect, and its interaction with osmoklinotaxis are described in this article.

Normal and experimental bees trained to a scented food source had to show in an appropriate experimental situation that they were able to distinguish between the scentless and the scent-filled branches of a Y-tube (Figs. 1*a* and *b*). At the moment of the decision they had the choice of running into one or the other of the tube branches in order to reach the food. The experimental set-up was free of air currents. The Y-tube was placed at an angle of 5°–6° to the horizontal plane. This guaranteed a flow of scent molecules (rate of flow: 0·3–1·5 cm/sec). Observation was through a ruby glass plate.

(1) *Bees with one movable antenna and with fixed antennae.* With the scent source on one side (0·002 ml. oil of anise), normal bees run straight to the scented box (Fig. 2). 2·5 sec were required to run a distance of 11·5 cm; 87·0 per cent positive choices were made. If one antenna is cut off the orientation towards the scent is scarcely impaired (78·8 per cent positive runs). Only occasionally a slight 'zig-zagging' can be observed. A tendency to move sideways[5,9,10] does not occur: time per run, 2·9 sec. If both antennae were fixed in their normal position

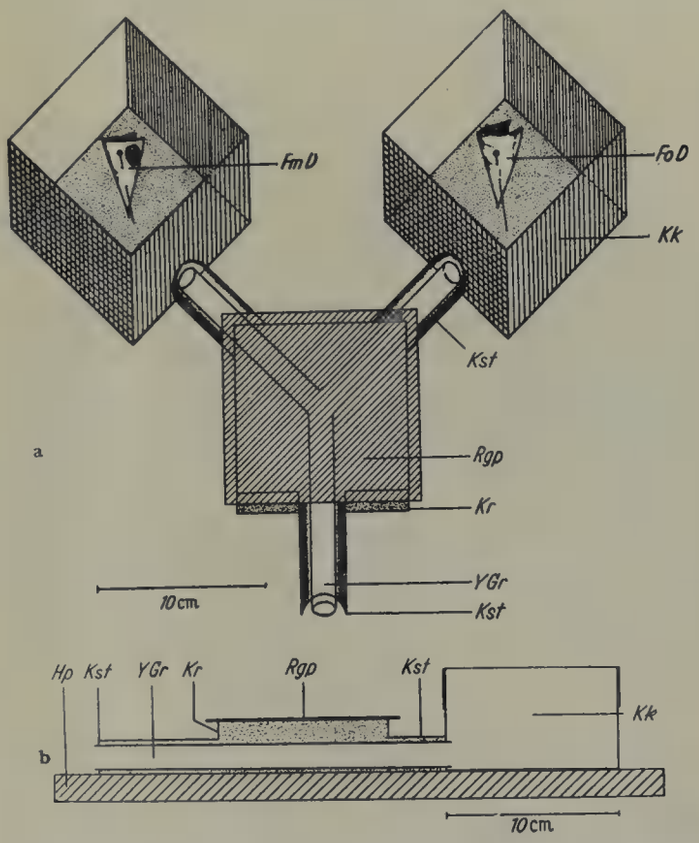


Fig. 1. *a*, Experimental arrangement for the Y-tube tests; *FmD*, filter paper with scent; *FoD*, filter paper without scent; *Kk*, cardboard box with lid removed; *Kr*, framework of the cardboard box (support for the ruby glass plate); *Kst*, box top (dark box); *Rgp*, ruby glass plate; *YGr*, Y-glass tube. *b*, Cross-section: *Hp*, fibreboard; *Kk*, cardboard box with lid; *YGr*, Y-glass tube; *Kr*, framework of the cardboard box; *Kst*, box top; *Rgp*, ruby glass plate

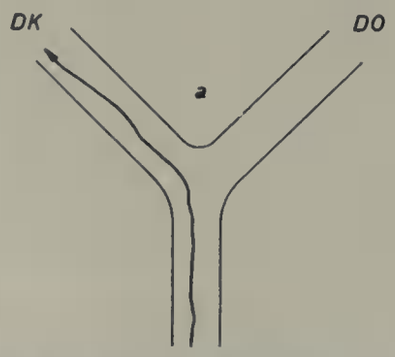


Fig. 2. Normal bee in the Y-tube (No. 157): without zig-zag movements the bee runs in a straight line towards the scented box. *DK*, scented box; *DO*, box without scent

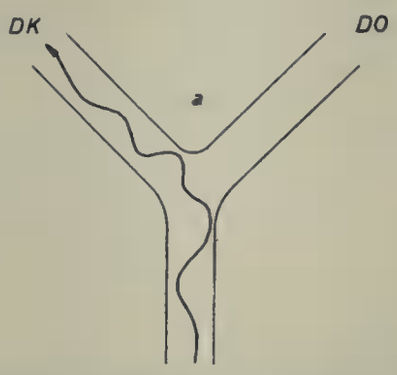


Fig. 3. Run of bee No. 108. Right antenna amputated, left antenna fixed on the morphologically correct side of the body. Zig-zagging run as indicator of klinotactic orientation

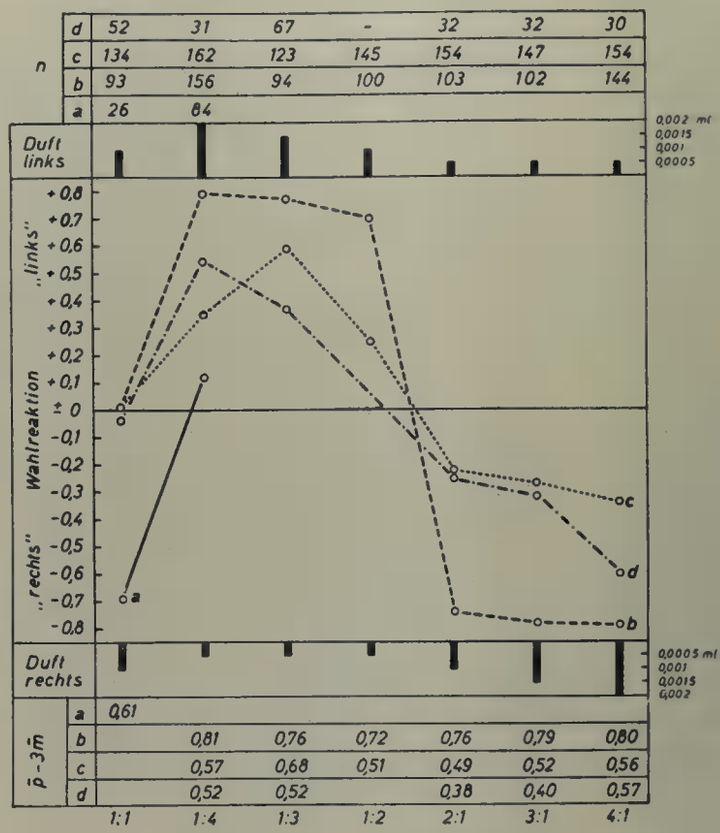


Fig. 4. Alternative choice of differently handled bees when different quantities of scent are offered simultaneously from the left and from the right. *a*, Bees with one fixed antenna (left antenna cut off); *b*, normal bees; *c*, bees with one movable antenna (right antenna cut off); *d*, bees with both antennae fixed in normal position

(distance between the tips of the antennae, 7–8 mm), then still 83 per cent positive choices were recorded. The run itself is a straight line; once in a while a slight zig-zagging appears: time per run, 3·4 sec.

Bees which have one antenna fixed and the other cut off tend to show a bias toward the intact side. The results in finding the objective vary from one condition to another: if the scent is given on the antenna side, then 76·9 per cent of these bees run into the tube branch with the scent. On the contrary, if the scent is offered on the amputated side, only 39·9 per cent of these bees run into the scented branch. The run to the scent source is a distinct zig-zag run (Fig. 3). Furthermore, the runs are appreciably slower (4·6 sec running time). One can show that the side tendency, which is frequently explained as an osmotropotactic reaction, is superimposed by a klinotactic orientation component. There is, therefore, a tendency toward the intact side. If scent is offered simultaneously and at different concentrations in the left and right tube branches, then normal bees, bees with fixed antennae, and bees with one movable antenna all choose the higher concentration (Fig. 4). The different scent ratios,

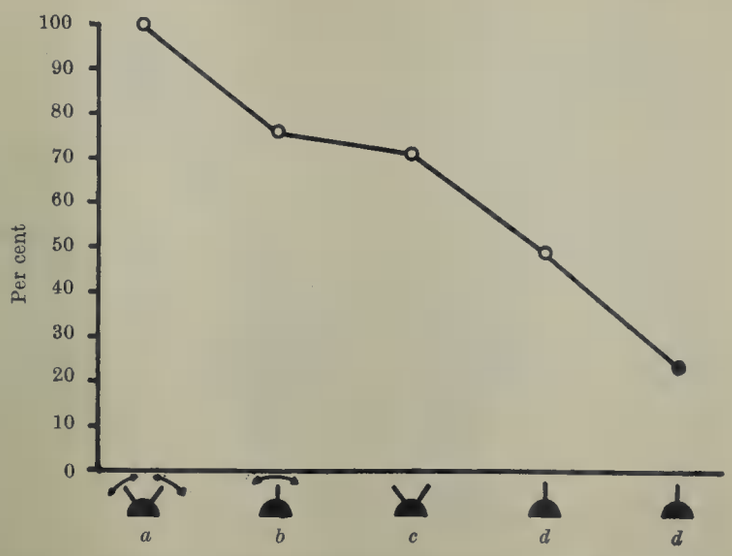


Fig. 5. 'Absolute' value in orientation (absolute orientation performance coupled with the time necessary finding the goal). The 'absolute' value in orientation for normal bees set at 100 per cent. *a*, Normal bees; *b*, bees with one movable antenna (the second antenna is cut off); *c*, bees with both antennae fixed in normal position; *d*, bees with one fixed antenna (the second antenna is cut off). ●, Bees with one fixed antenna: scent is offered on the side without an antenna. ○, bees with one fixed antenna: scent offered on the antenna side

however, are analysed with varying success by the different bee groups (Fig. 4). Bees with one fixed antenna and the other antenna cut off compensate for the side tendency only, if the scent ratio is 1 : 4 and when the greater concentration is given on the side without antenna (Fig. 4, curve *a*).

This method allows one not only to separate the side tendency and its connected klinotactic orientation component from one another, it also gives the first hint of the integration process in the central nervous system. The scent gradient between the left and the right side must be 1 : 4, so that a difference in the information from two sides will be recorded in the central nervous system. This is effective for cases in which the klinotactic component is experimentally removed from the orientation act and only the tropotactic adjustment is allowed (experiments on bees with both antennae fixed in normal position). The same holds also for the information from one side when the tropotactic component is eliminated and only klinotactic adjustment is allowed (experiments on bees with one antenna free). By a combination of tropotactic and klinotactic adjustment the central nervous system can detect smaller concentration differences (experiments on normal bees being about a ratio of 1 : 2).

Olfactory perception of the attracting scent therefore consists of two components: perception of the concentration gradient simultaneously in space and successive perception in time. The simultaneous component demands paired, symmetrically arranged receptors—at least two; they do not need to be movable. The successive component requires movement of the sense organ (theoretically one receptor would be enough) or movement of the animal. Normal bees use both these components in coupled ways. Bees with one antenna can only use successive perception of the concentration for orientation. The net performance in scent orientation (calculated in percentages) is almost equal for normal bees (87·0 per cent), for bees with one movable antenna (78·8 per cent), and for bees with fixed antennae (83·8 per cent). However, the individual groups approach the goal with different velocity ($P < 0{\cdot}0027$). The net performance in scent orientation coupled with this time factor gives the 'absolute' value of orientation (Fig. 5). It shows us quite clearly the true grade of performance in olfactory orientation.

(2) *Crossing the antennae.* The antennae of bees which were trained to odorants are crossed at the scapus and fixed into this position (Fig. 6). By this crossing, the instinctive, natural side-correct evaluation of information is no longer given. Bees with crossed, fixed antennae turn spontaneously and without hesitation at the point of decision into the scentless tube branch. This 'changing of polarity' of the antennae causes the olfactory information in the central nervous system to be recorded as reversed. It also causes efferences to be sent out side-reversed. This way brings about the 'spontaneous turning' into the tube branch without scent. Their behaviour shows that they perceive the scent gradient spatially simultaneously between the antennae.

If the tips of the symmetrically crossed antennae are placed closer together, step by step along the median line, then a change from osmotropotaxis to klinotaxis occurs when the distance between the tips of the antennae is 2·0 mm (Table 1). This reaction is quite definite. If the distance is smaller than 2·0 mm, then the bees turn into the tube branch with the scent and make a zig-zag run to the scented box. This means, therefore, that simultaneous-spatial distinctions are no longer made, and only temporal sequences can be evaluated. Quite astoundingly, in antenna positions I–V, almost the same number of 'side-reversed correct' choices are made (see Table 1). The change from one orientation type to the other does not happen gradually, but rather suddenly. With asymmetrical crossing of the antennae (Fig. 7) the 'effective' boundary distance totals 2·7 mm.

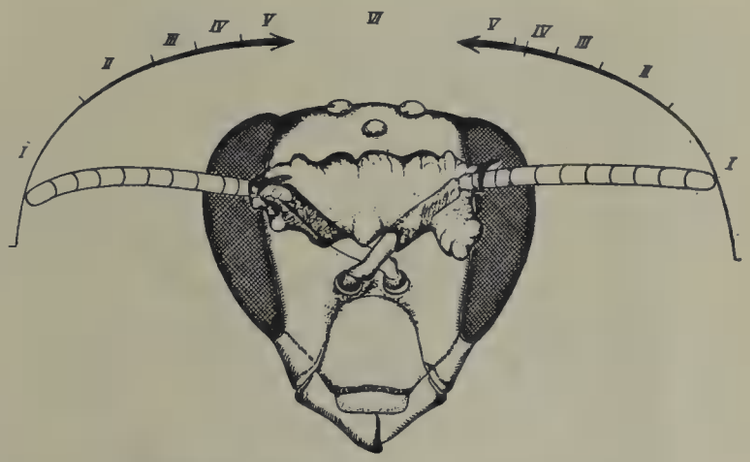


Fig. 6. Bee head with symmetrically crossed and fixed antennae. The sections I, II, III, IV, V and VI give the possible position of the antennae during the experimental series. If the antennae are in position VI, then the bees orient themselves klinotactically; in all the other positions, osmotropotactically

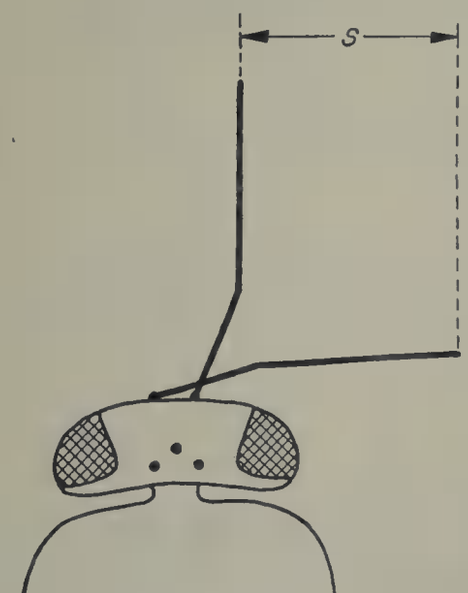


Fig. 7. Schematic representation of a bee head with asymmetrically crossed antennae. Both antennae are fixed on one morphological side of the body. *s*, 'Effective' distance between the antennae tips

Table 1. Choices for the Branches of the Y-tube from Bees the Antennae of which are glued and switched to the Opposite Side at Various Different Angles

	Scent from the right (0·002 ml. anise oil					
Position of the crossed antennae	I	II	III	IV	V	VI
'Correct', side-reversed choices (%)	79·9	76·6	72·4	77·5	74·3	23·4
Total number of choices	194	205	181	111	202	124
Distance between the antenna tips in mm	9–7	7–5	5–4	4–3	3–2	2–0

Osmoklinotactic orientation behaviour appears also when one antenna is fixed and the second antenna is left free, provided that the 'median distance' of the fixed antenna (Fig. 8) is smaller than 2·5 mm. The movable antenna can compensate for the false report of the crossed, fixed antenna only so long as the 'median distance' of the second antenna does not exceed 2·5 mm (Fig. 9).

(3) *Quantitative effect of osmotropotaxis.* How great must the difference of concentration between the left and the right antenna be in order to be perceived osmotropotactically?

Fine glass capillaries of a certain weight and diameter are slipped over the antennae and are glued on the scapus (Fig. 10). (The bees are previously trained to the scent.)

Liquid paraffin with scent of different concentrations is placed in the capillaries to produce different scent

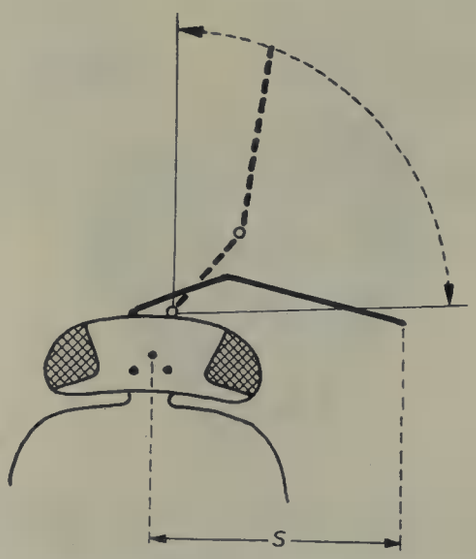


Fig. 8. Schematic representation of a bee head with one fixed and one movable antenna. Left antenna fixed on the right side of the head. Right antenna movable. *s*, Median distance (equals the projected, perpendicular distance in the horizontal plane between the stirnocellus and the tip of the fixed antenna

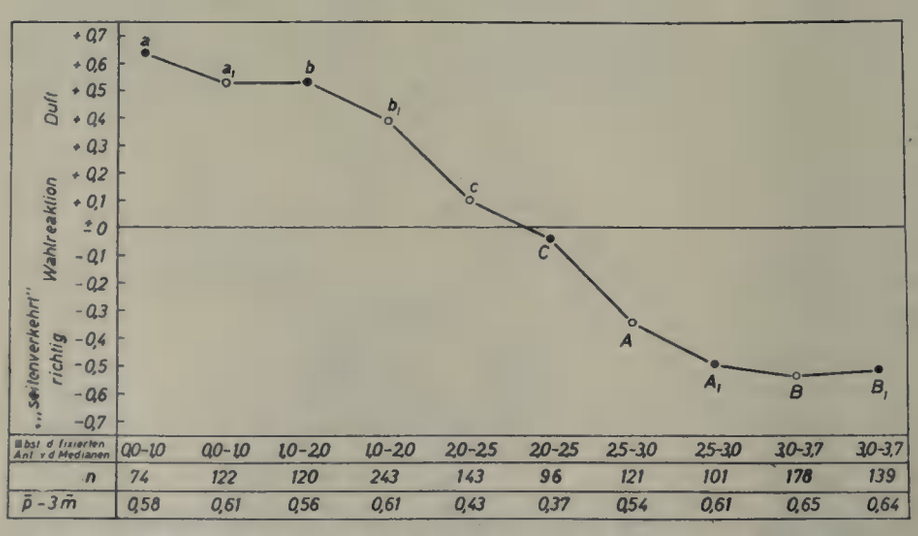


Fig. 9. Reaction curve of bees with one antenna fixed on the opposite side (with different-sized median distances) while the other antenna remains movable. Scent from one side (0·002 ml. oil of anise). Distance of the fixed antenna from the median line is given in mm. ●, Values for those which received the scent on the side without antenna; ○, values for those which received the scent on the antenna side

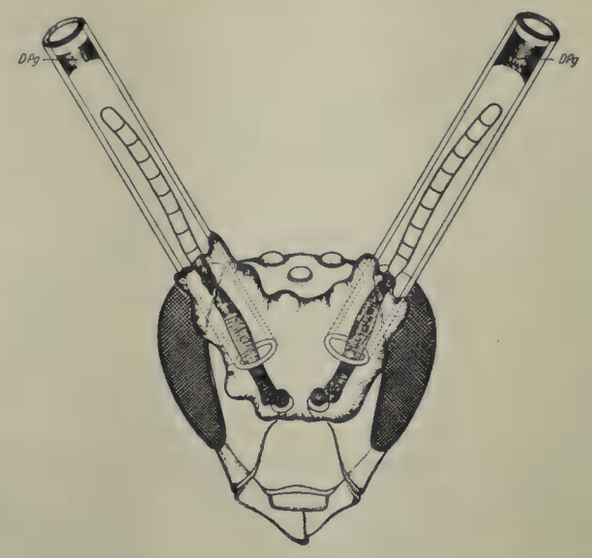


Fig. 10. Bee head with glass tubes slipped over the antennae. The scapus-head joint is blocked. *DPg*, Mixture of liquid paraffin with scent

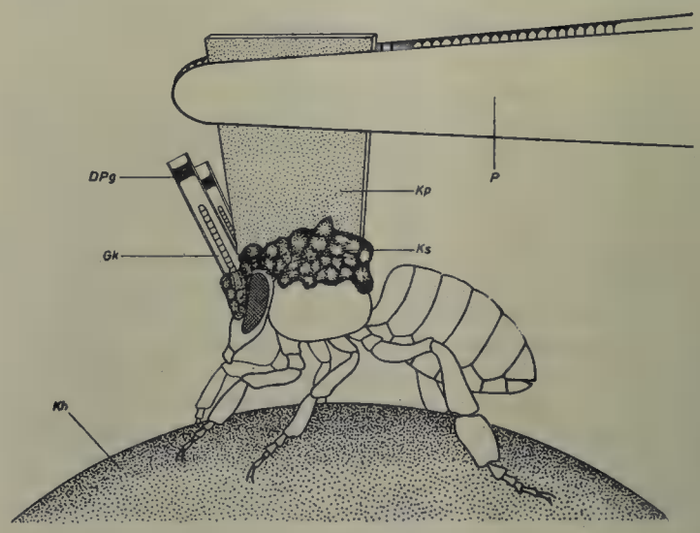


Fig. 11. Experimental bee stuck on to the cork hemisphere. Only a part of the cork hemisphere, the 'running area', is illustrated. *DPg*, Mixture of liquid paraffin with scent; *Gk*, glass capillaries; *Kh*, cork half-sphere; *Kp*, pieces of cardboard; *Ks*, glue; *P*, pincers

recognition reactions by the two antennae. The insect in the experiment is placed on a movable cork hemisphere (diameter, 4 cm) and is held down by means of a small plate on its thorax (Fig. 11). Direction and strength of the reaction are read off by means of a pointer and a scale (Fig. 12). So long as a difference between scent concentrations in the right and the left capillaries can be perceived, a directed run towards the more strongly scented side occurs. That means that the hemisphere turns exactly opposite to the direction in which the animal would turn if it were not glued down. Therefore, if the higher concentration is found in the right capillary, then the animal tries to compensate for the difference of concentration by turning to the right; that results in the cork hemisphere being turned to the left.

The lower concentration, 'initial' concentration, is held constant in all experiments. The 'test' concentration is varied for each test (a turn of 90° is given the absolute value of 1).

With high initial concentrations (1/10,000) the osmotropotactic ability to discriminate different scent concentrations is of the ratio 1 : 10 (Fig. 13). The change to

osmoklinotaxis occurs gradually at this concentration level (Fig. 13, curve *a*). With low initial concentrations (1/100,000), the ability to analyse different scent concentrations is at a ratio of 1 : 2·5 (Fig. 14). Here the change to klinotactic orientation occurs suddenly (Fig. 13, curve *a*). Therefore the osmotropotactic threshold is not an absolute value, but rather it is a variable depending on the initial concentration. This is biologically significant for the evaluation of different scent zones through which a bee moves when it is approaching a scent source. It is especially shown in these experiments that the olfactory perception of scent differences can operate basically without anemotaxis. In Nature, of course, anemotaxis works along with osmotropotaxis.

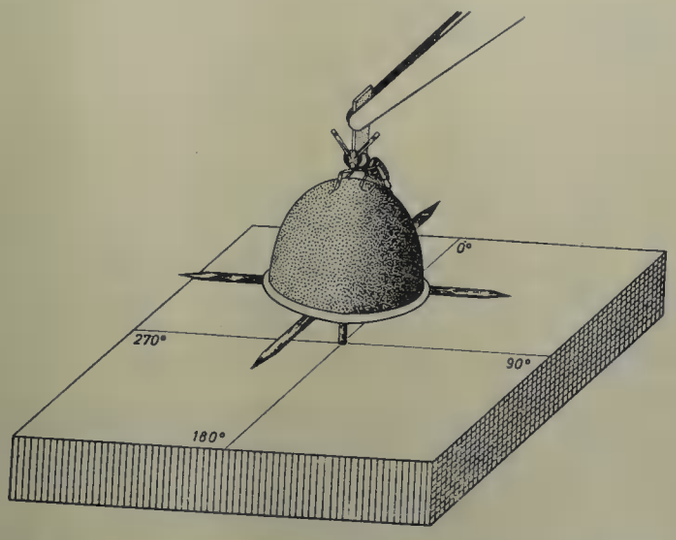
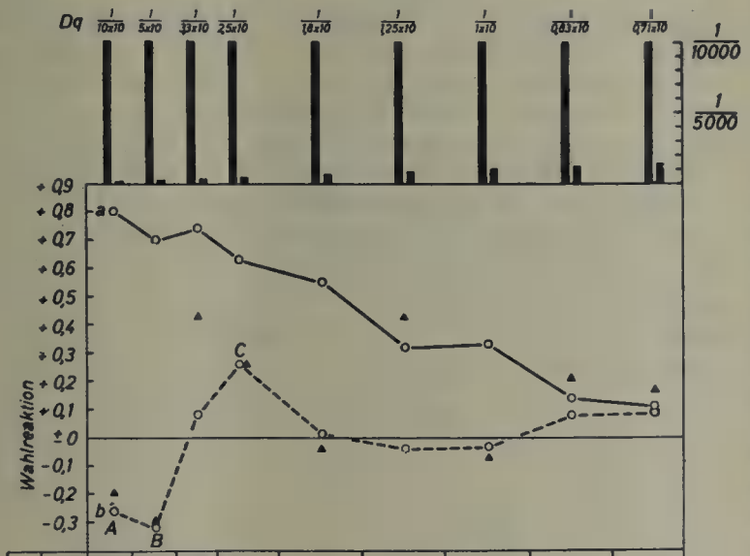
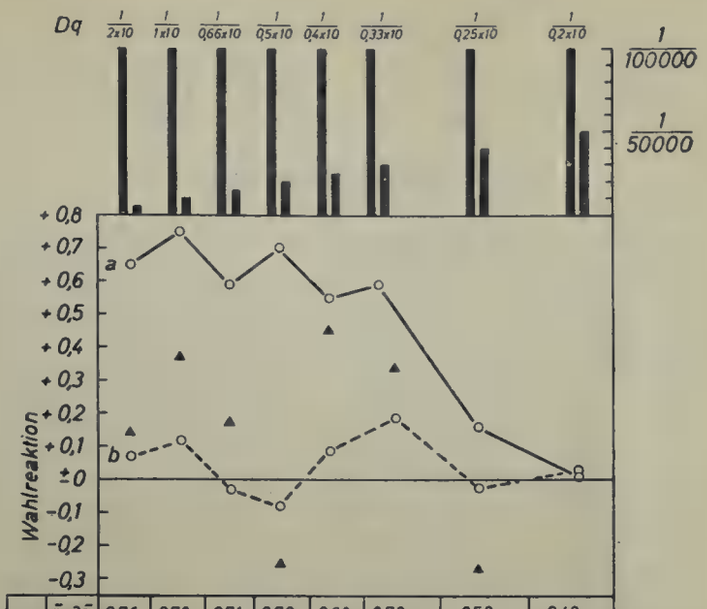


Fig. 12. Representation of the experimental arrangement for determining the osmotropotactic threshold in the scent field. The cork hemisphere (3·1 g weight) can be turned almost without resistance. With the help of the attached pointer, the direction and the strength of the turning tendency per unit of time can be read off. A turn of 90° of the cork hemisphere—the direction does not matter—gives the relative value of 1



a	$\tilde{p}$-3$\tilde{m}$	0,86	0,77	0,78	0,67	0,67	0,55	0,52	0,44	0,39
	n	455	234	153	80	157	226	105	315	380
b	$\tilde{p}$-3$\tilde{m}$	0,57	0,58	0,45	0,53	0,42	0,40	0,46	0,46	0,46
	n	615	364	252	100	255	375	962	316	377
c_A	$\tilde{p}$-3$\tilde{m}$	0,50	0,55	0,59	0,47	0,38	0,62	0,27	0,52	0,49
	n	198	222	154	81	109	226	56	184	237

Fig. 13. The determination of the osmotropotactic threshold with high initial concentration (1/10,000). Scent oil: methylheptenone. The black columns give the concentration ratios in both capillaries. *a*, Values from 0–3 min calculated from the beginning of the experiment; *b*, corresponding values in the 5–10 min period; *c*, ▲, values of 3–5 min. In the 3–5 min period the osmotropotactic reaction ends. The osmotropotactic threshold with high initial concentrations is at a concentration ratio of 1 : 10 between both antennae. *Dq*, Scent gradient quotient



a	$\tilde{p}$-3$\tilde{m}$	0,76	0,78	0,71	0,79	0,68	0,70	0,52	0,42
	n	360	181	333	214	220	229	413	275
b	$\tilde{p}$-3$\tilde{m}$	0,45	0,47	0,41	0,42	0,43	0,47	0,43	0,41
	n	352	241	486	209	141	151	365	239
c_A	$\tilde{p}$-3$\tilde{m}$	0,48	0,56	0,50	0,49	0,59	0,46	0,55	0,35
	n	252	134	220	117	99	118	231	92

Fig. 14. Determination of the osmotropotactic threshold with low initial concentration (1/100,000). Scent oil: methylheptenone. The osmotropotactic threshold with lower initial concentrations is at a concentration ratio of 1 : 2·5 between the antennae. The remaining explanation is the same as for Fig. 13

Noted also was that the determination of thresholds can be carried out very well on the basis of the osmotropotaxis (Table 2).

Table 2. Determination of the Level of Methylheptenone and Bromstyrol

For both substances the threshold is at a concentration of 1 : 200,000

Concentration	Methylheptenone		Bromstyrol	
	Scent choices (%)	0–5 min after releasing the scent (No.)	Scent choices (%)	0–5 min after releasing the scent (No.)
1 : 50,000	83·0	697	90·5	154
1 : 100,000	88·5	637	85·5	513
1 : 200,000	73·4	672	71·0	888
1 : 300,000	50·8	859	58·1	931

These experiments have demonstrated that osmotropotaxis together with osmoklinotaxis are of vital importance in the rich mosaic of orientation behaviour of bees.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Sky Brightness at 14·1 Mc/s

Knowledge of the intensity–frequency relation of extraterrestrial radio emission has been obtained from scaled aerial experiments at frequencies higher than 20 Mc/s (refs. 1 and 2) and from ground-based or satellite-borne radiometers operating below 10 Mc/s (refs. 3, 4 and 5). The low-frequency observations, whether ground-based or satellite-borne, are affected by the ionosphere. The consequence of these ionospheric effects has resulted in difficulties being experienced when reconciling the observations in frequency regions below 10 Mc/s with those above 20 Mc/s.

It has now been possible to extend the scaled aerial measurements of sky temperature down to a frequency of 14·1 Mc/s. The present sunspot minimum resulted in low values of the ionospheric critical frequencies, allowing unhampered night-time observations at this frequency. The frequency of the present observations is close to those used in satellite experiments of Hartz[4] and Smith[5], which provide good values of relative intensities below 10 Mc/s. No highly reliable absolute values of sky temperature were provided in these experiments. By joining the satellite results with our present 14 Mc/s results and our previous scaled aerial measurements, a curve of the sky brightness–frequency dependence is obtained for the frequency region 1–100 Mc/s.

The 14·1 Mc/s observations were made with a low-resolution aerial directed at the zenith ($\delta = -34°$) which contains, at different times, the galactic centre, the disk and the south galactic pole. At 0800 and 1700 h R.A. 60 per cent of the radiation received originated from galactic latitudes $-20° < b < 20°$, while no radiation was received from this belt between 2130 and 0400 h R.A. The south galactic pole was in the centre of the beam at 0040 h R.A.

The aerial used was scaled from those previously used by us at frequencies 20, 30 and 85 Mc/s. An additional result was obtained at the frequency of 48·5 Mc/s.

The aerial consisted of a single half wave-length folded dipole suspended 0·25 λ above a continuous mesh screen a wave-length square in area. The receiver was a switched radiometer using a *B40B* communications receiver preceded by a low-noise pre-amplifier of 75 Ω characteristic impedance. Calibration, which consisted of rematching the aerial and replacing it by a noise generator, was performed each hour. The noise generator used consisted of two *CV2398* noise diodes in parallel, working into a 75 Ω load. The maximum temperature available from this generator was 80,000° K. This is well below the sky brightness temperature at 14·1 Mc/s. It was thus necessary either to amplify the noise signal or else attenuate the sky signal. Both methods were tried and found satisfactory. In practice, a 9 dB calibrated attenuator was inserted between the aerial and the receiver. The accuracy of the noise calibration was checked by comparing two different generators. The two systems reproduced calibrations to within 2 per cent.

Ionospheric effects are serious obstacles to reliable absolute measurements at this frequency. The two separate effects that need to be considered are absorption in the *F* layers and the 'iris' effect. All observations were carried out at night, thus avoiding *D* layer absorption. Absorption in the *F* layers has been theoretically investigated by Ellis[6], using a model based on thermal loss by collision of electrons with ions and neutral atoms. The theoretical results obtained agreed with extinction curves determined by Mitra and Shain[7] and Steiger[8] at 18 Mc/s. From our own results and ionospheric data from Camden, 15 km distant, it was possible to plot an extinction curve for each hour of R.A. At least five calibrated measurements were available for each hour spaced over 6 h local time. During the observing period, December 1964 to July 1965, the night-time values of f_0F_2 were almost always less than 4 Mc/s. This limited the extent of the extinction curves. For $f_0F_2 = 4$ Mc/s, absorption was observed to be less than 0·1 d*B*, agreeing with theory. The 'iris' effect produces total reflexion of galactic emission for zenith angles:

$$\chi \geqslant \cos^{-1} \frac{f_0F_2}{14{\cdot}1 \text{ Mc/s}}$$

At such angles the Earth temperature of 300° K is reflected into the aerial beam. A calculation was made of the attenuation due to the iris effect and it was found to be negligible for f_0F_2, less than 6 Mc/s.

In addition to correcting for the ionosphere an allowance was made for losses in the aerial system which amounted to 0·1 dB in the matching transformer and 0·1 dB in the balun. The mesh reflecting screen was assumed to be perfect.

Fig. 1 shows the observed sky temperatures as a function of right ascension for $\delta = -34°$, after the foregoing corrections had been made. We note that the minimum occurs at 0400 with $T_B = 110{,}000°$ K. At 0040 h R.A., when the south galactic pole is in the main beam, $T_B = 125{,}300°$ K. Because of the low resolution of the aerial, temperatures observed when there is a sharp gradient of sky temperature in the main beam have little meaning and thus we have omitted results from 1600 h to 2000 h R.A. Overall accuracy is better than ± 7 per cent.

Fig. 2 shows the present flux measurement for the south polar region, together with other scaled aerial values and the two satellite observations. Absorption in ionized hydrogen regions is a minimum in the direction of the galactic poles, and thus Fig. 2 gives a good estimate of the synchrotron emission spectrum. We note that at 16 Mc/s the temperature spectral index β, ($T \propto \lambda^{\beta}$), has fallen from its high frequency value of 2·65 to 2·55 ± 0·15.

Some caution needs to be observed when interpreting these results, since the observed sky temperatures include an isotropic extragalactic component superimposed on the galactic emission. The spectrum of this extragalactic component is thought to be steeper and thus would dominate at low frequencies. Using the scaled aerial results it should be possible to separate the galactic and extragalactic components if the two components

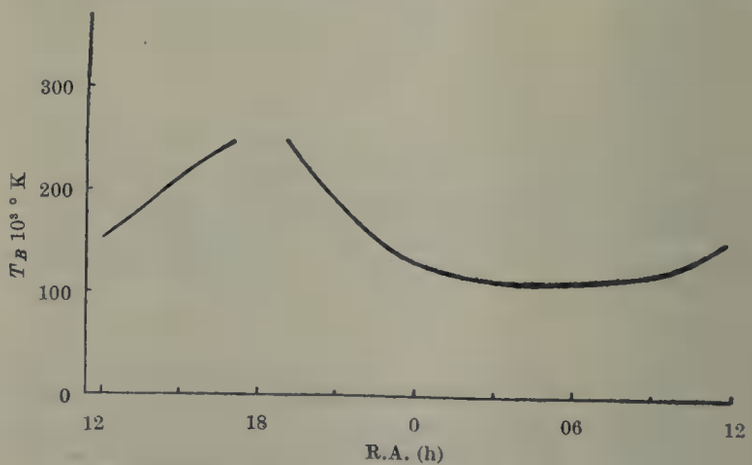

Fig. 1. Observed sky temperatures at 14·1 Mc/s, $\delta = -34°$

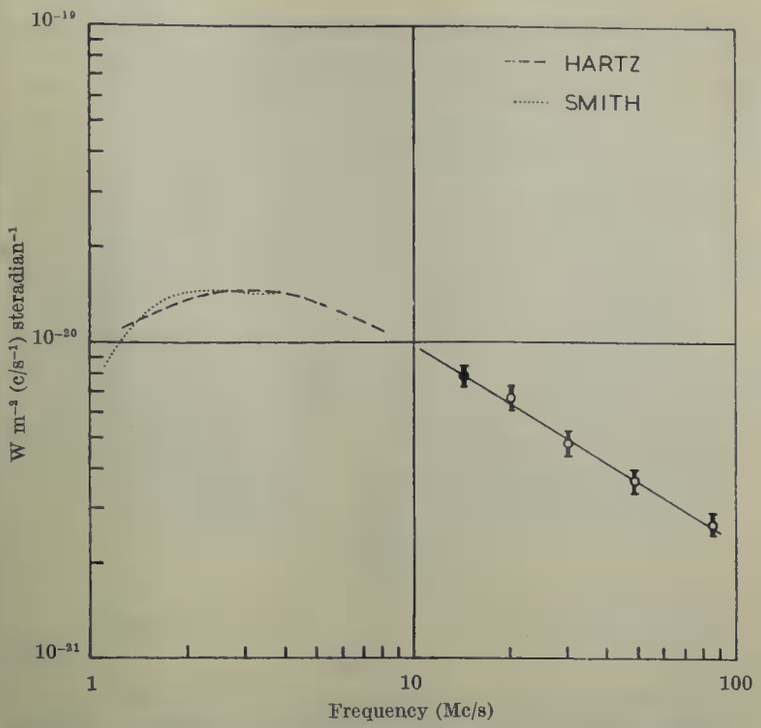

Fig. 2. Brightness-frequency relation. Scaled aerial results above 10 Mc/s, satellite results below 10 Mc/s

have differing spectra. The separation will be discussed by us elsewhere.

We are now making a new series of observations with scaled aerials of medium resolution.

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ASTROPHYSICS

Pulsation Periods of General Relativistic Objects

The pulsation period of the lowest radial mode for a spherical homogeneous object in Newtonian theory is[1] (if $\Gamma_1 = \text{const}$):

$$\tau = 2\pi[4\pi G\rho(\Gamma_1 - 4/3)]^{-1/2}$$

and if $\Gamma_1 - 4/3 > 0$, the period may vary over a wide range depending on the density.

However, a spherically symmetric homogeneous object in the post-Newtonian approximation has a period of[2]:

$$\tau = 2\pi c\left\{4\pi G\varepsilon(\Gamma_1 - 4/3)\left[1 - (GM/c^2R)\left(\frac{10}{7}\Gamma_1 - 1\right)\right]\right\}^{-1/2}$$

where ε is the total energy density. From this equation it is easily seen that there will be a minimum period even if $\Gamma_1 - 4/3$ is always positive and finite. Substituting:

$$\varepsilon = 3Mc^2/4\pi R^3$$

we obtain:

$$\tau = 2\pi\left\{GMR^{-3}(3\Gamma_1 - 4)\left[1 - (GM/c^2R)\left(\frac{10}{7}\Gamma_1 - 1\right)\right]\right\}^{-1/2}$$

A very massive star (superstar) has a Γ_1 which is a function of mass and is approximately 4/3. The quantity $\Gamma_1 - 4/3 = \alpha(M/M_\odot)^{-1/2}$ (refs. 3 and 4). The minimum period will therefore occur at a radius of:

$$R = 76 \times GM/[63(\Gamma_1 - 4/3)c^2]$$

and:

$$\tau_{\min} = (304 \times \pi GM/63c^3)\,(76/189)^{1/2}(\Gamma_1 - 4/3)^{-2}$$
$$= 4{\cdot}73 \times 10^{-5}\alpha^{-2}(M/M_\odot)^2 \text{ sec}$$

or:

$$\tau_{\min} = 1{\cdot}50 \times 10^{-12}\alpha^{-2}(M/M_\odot)^2 \text{ yr}$$

A superstar of $10^6\ M_\odot$ would therefore have a period of (for $\alpha = 1{\cdot}4$) 0·77 yr. If quasi-stellar sources are very large massive objects with fluctuations of the order of years, then one would not expect them to be much larger than $10^6\ M_\odot$ because of the quadratic mass dependence of the periods. W. A. Fowler has recently pointed out that rotation may enable one to have smaller periods and therefore smaller radii[5].

This general relativistic effect of a minimum period also manifests itself in neutron stars[2] and white dwarfs, although in these cases the models are not homogeneous and Γ_1 is not constant.

I thank A. D. Code for his advice.

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Angular Momenta of Eclipsing Binaries and the Fission Theory of their Origin

In a new development of the fission theory of the origin of close binary stars[1,2], I showed that rotational instability would occur during the pre-main sequence contraction of rotating stars with no internal magnetic field. The theory predicted the observed mass range for contact binaries of *W*-Ursae Majoris type with satisfactory accuracy, and also gave the observed variation of angular momentum with mass for these systems. I now wish to show that the theory also predicts the observed relation between angular momentum and mass for all the close binary systems.

The rotational instability which gave rise to the formation of binary systems was due to the different behaviour of convective and radiative regions of stars. When the star is fully convective, as it is during early stages of contraction[3], it rotates uniformly, and as the angular velocity increases the star spins of mass at the equator, and the effect of rotation is small over the bulk of the star. When the star begins to develop a radiative core, the rotation is no longer uniform as each element of the radiative core contracts, conserving its angular momentum. The parameter that measures the effect of rotation is:

$$\alpha = \frac{\Omega^2}{2\pi G\rho_c} \qquad (1)$$

where Ω is the angular velocity and ρ_c the density at the centre of the star. When the star was fully convective, then with uniform rotation and centrifugal force balancing gravity at the surface, $\alpha = 0{\cdot}04$ (ref. 4). With the development of the radiative core α varies like $\rho_c^{1/3}$ and so it will reach the critical value for instability, 0·187 (ref. 5), when ρ_c has increased by a factor 100.

The increase in ρ_c is due to two effects : the changing degree in central condensation due to the transition from convective energy transport, $\rho_c/\bar{\rho} = 6$, to radiative energy transport, $\rho_c/\bar{\rho} \simeq 24$ for massive stars and 54 for small stars, and the increase in the mean density $\bar{\rho}$ due to the decrease in radius. As the readjustment in internal

structure takes place during a small change in radius, the central density will have increased to its critical value when the radius of the star has decreased by a factor of 2·3 for small stars and 3 for large stars.

The angular momentum of the star at the onset of rotational instability can be estimated. With centrifugal force balancing gravity, at the end of the fully convective stage the angular momentum is:

$$H_H = K\,G^{1/2}\,M^{3/2}\,R_H^{1/2} \qquad (2)$$

where R_H is the radius of the star and $K^{1/2}\,R_H$ its radius of gyration. For a fully convective star $K \simeq 0{\cdot}13$ (ref. 4). During the fully convective phase it is known that the star has an approximately constant surface temperature $\simeq 4{,}000°$ K. The radius R_H at which convection begins to die away can then be computed by equating the energy carried by radiation to the release of internal energy during the collapse of a fully convective star. This gives:

$$\lim_{r\to 0}\left[-\frac{4ac}{3}\frac{T^3}{\varkappa\rho}\frac{1}{r}\frac{dT}{dr}\right] = \frac{7}{6}\frac{LP_c}{GM^2}R \qquad (3)$$

where T is the temperature, $\varkappa$ the opacity, P_c the value of the pressure at the centre, a the radiation constant, c the velocity of light, L the luminosity $= \pi acR^2T_e^4$ (where $T_e \simeq 4{,}000°$ K). With the opacity $\varkappa$ known as a function of pressure and density, and the temperature and pressure known in terms of the mass and radius for a fully convective star[6] (a polytrope $n = 1{\cdot}5$) this is an equation to determine R_H, and hence from equation (2) H_H is known. Knowing the decrease in radius to the onset of instability we can get a lower bound on the angular momentum at the onset of instability, H_I, by neglecting the growing radiative core; an upper bound is obtained by assuming all the star is radiative. The actual value H_I must be somewhere between these limits, and we take the arithmetic mean[1,2]. Since the decrease in radius to the onset of instability is at most by a factor of 3, and as the angular momentum varies like $R^{1/2}$ (equation 2) the lower bound on H_I is only less than H_H by at most 0·23 in the logarithm.

In Fig. 1 the predicted relation between angular momentum and mass of rotating stars at the onset of fissional instability is plotted, and also the observational values for all the close binary systems with spectroscopically determined mass ratios that are given in Kopal and Shapley's catalogue[7], except for the few that are too massive to fit on our diagram. The agreement is very satisfactory.

The alternative theory of binary star formation, the capture theory, is unable to explain the observed correlation between mass and angular momentum, since the capture process is a chance phenomenon due to the close approach of two bodies in the presence of a third. There is no reason to suppose that this would give anything other than a random distribution of masses and angular moments. The observed relation between mass and angular momentum lends support to the fission theory, and it is very satisfactory that the observations and predictions agree so well.

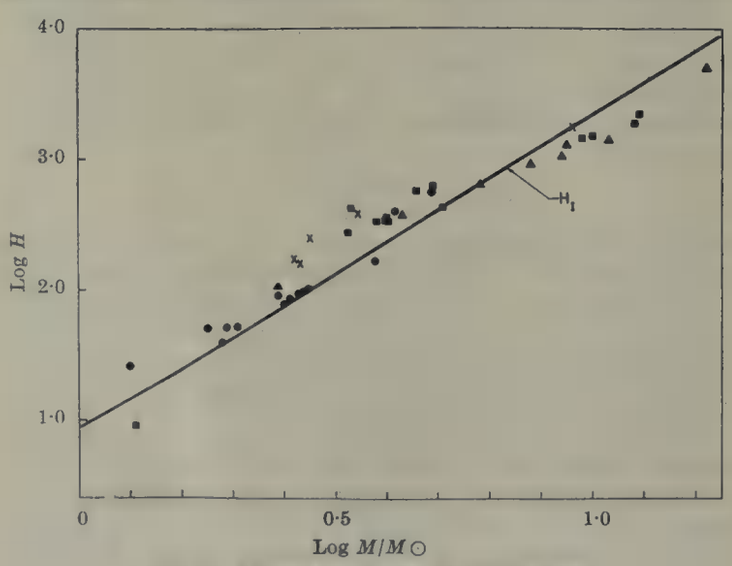

Fig. 1. Angular momentum of close binaries

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PHYSICS

Laser-induced Damage in Natural White Diamond

A previous report[1] has described laser-induced damage in optically transparent materials such as glass, alkali halides, CaF_2, MgO, sapphire and quartz; we wish to report the observation of this effect in natural white diamond. Intense, 0·3 joule, 10 MW pulses of 6943 Å radiation from a kryptocyanine Q-switched ruby were focused with a 5-cm focal-length lens on to the polished surfaces of the specimens. The dimensions of the specimens were very much smaller than the focal length of lens, thus ensuring that the power density of 10^9 W/cm² incident on the surface was constant throughout the specimen. The damage on the front surface was characterized by a seared mark apparently consisting of black carbon (Fig. 1), which indicated that a high surface temperature was attained. The rear surface damage was characterized by extensive fractures and cracking (Fig. 2). Because of the difficulty in sectioning diamond, it was not possible to determine the depth of the physical damage; however, as this was not observed on the front surface of the specimens it seems apparent that it is characteristic of the rear surface only. This is consistent with the observed damage in other optically transparent materials[1].

It has been proposed[1] that this damage is a result of acoustic phonons which travel in the forward direction through the material. These phonons are generated by stimulated Brillouin scattering of the laser light, and this process is similar to Raman laser action with the molecular vibration replaced by an acoustic wave. Other

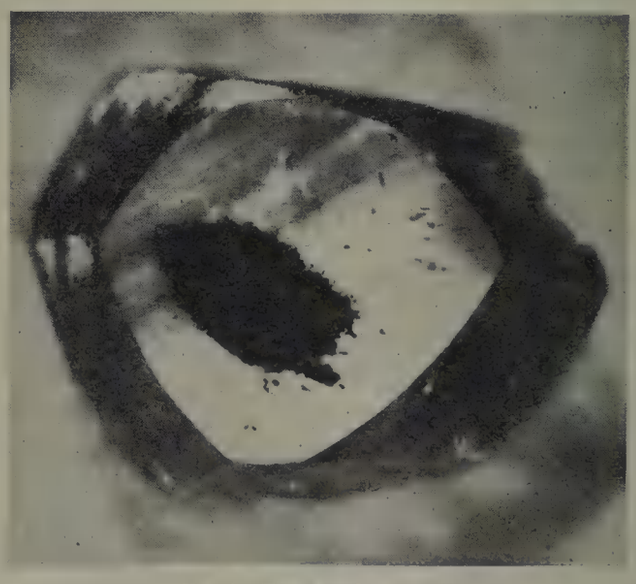

Fig. 1. Front surface (× 16)

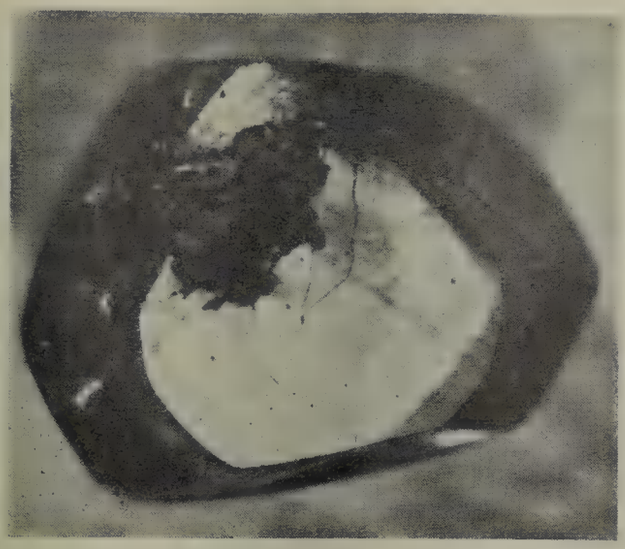

Fig. 2. Rear surface (×16)

damage mechanisms, such as direct heating from optical absorption and the interaction of light with material boundaries, were eliminated by detailed experiment. The acoustic phonon damage mechanism is supported by direct measurements of the frequency of the scattered radiation from the stimulated Raman process for quartz and sapphire[2].

The frequency of the acoustic phonon wave f is[2] $f=\frac{2f_1vn}{c}$ where f_1 is the frequency of the incident light which is about 4×10^{14} c/s for ruby light, v is the velocity of sound in the material of refractive index n, and c is the velocity of light. For diamond, n is 2·4, and from measurements of the compressibility[3] v is calculated as $1{\cdot}8 \times 10^6$ cm/sec, whence f is about 120 Gc/s. This compares with 60 Gc/s for sapphire and 12 Gc/s for glass. The power in the acoustic wave is found from the ratio of the phonon to photon energies and is about 10^{-4} of the incident power or about 100 kW/cm² in these experiments.

These experiments were performed in an attempt to use a pulsed solid-state laser for the drilling of small holes in diamond. The results which we obtained show that the desired holes were not obtained because of the nature of damage mechanism.

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Newcastle upon Tyne, 6.

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Re-entrant Hysteresis Loop and Multistable Ferrites

It is well known that certain ferrites, especially CoFe ferrite[1], become magnetically anisotropic when heated in a magnetic field. This treatment results in an increased remanence ratio (I_{rem}/I_{sat}) and a square hysteresis loop. The explanation in terms of an ionic diffusion ordering process has been well developed[2]. It is also well known[3] that annealing in a unidirectional field results in a loop which appears displaced along the H-axis, but no adequate explanation has been given for the phenomenon in these materials. Glaister and Viney[4] have reported re-entrant hysteresis loops in these materials, and offered a tentative explanation for this phenomenon.

We have now prepared ferrite toroids with multistable remanent states in addition to re-entrant loops, and propose an explanation both for these effects and for the asymmetry resulting from a unidirectional field anneal. The material comprises $Co^{2+}_{0{\cdot}15}Fe^{2+}_{0{\cdot}85}Fe^{3+}_2O_4$ and is sintered under conditions that result in slight oxidation of Fe^{2+} content; this deficiency yields cation vacancies and thus greatly increased mobility[5] for the Co^{2+} ions responsible for anisotropy. On cooling, these ferrites show constricted or 'perminvar' loops. The samples are annealed in alternating fields of amplitude just sufficient to achieve technical saturation at 200° C and slowly cooled with the field applied. A very square re-entrant loop may then be observed (Fig. 1*a*) with a display system in which the magnetizing field is sensitive to the rate of change of flux[4].

The flux reversal (switching) time of a square loop ferrite is equal to the quotient of the average distance traversed by the domain walls and the wall velocity. Thus the number of domain walls that participate in flux reversal is proportional to the reciprocal product of the domain wall velocity and the switching time (or wall mobility and switching constant). An estimate of this wall mobility using the treatment of Menyuk and Goodenough[6] (3,400 cm sec^{-1} Oe^{-1}) and measurement of the switching constant (500 μs Oe) for a toroid with 4-mm wall section suggests that it is unlikely that more than one wall is involved in the flux reversal. Pulse measurements confirm that the rate of change of flux reversal is almost constant so that the comparison is valid.

Although samples with pronounced re-entrance are bistable and with slowly varying fields switch directly from one near-saturation state to the other, it is possible by applying single-shot pulses of about 2 μs duration to bring the domain wall to a central position giving zero net remanence. If the sample is then annealed in this state[7] at 200° C a loop of the form shown in Fig. 2*a* is obtained; the zero-magnetization state has become stabilized and a field of 26 Oe in excess of the wall coercive field (20 Oe) is required to free the domain wall. Such cores have three stable states. Evidently the component of the field within the Bloch domain wall directed along the toroid axis has resulted in localized anisotropy with an easy axis perpendicular to the general circumferential direction.

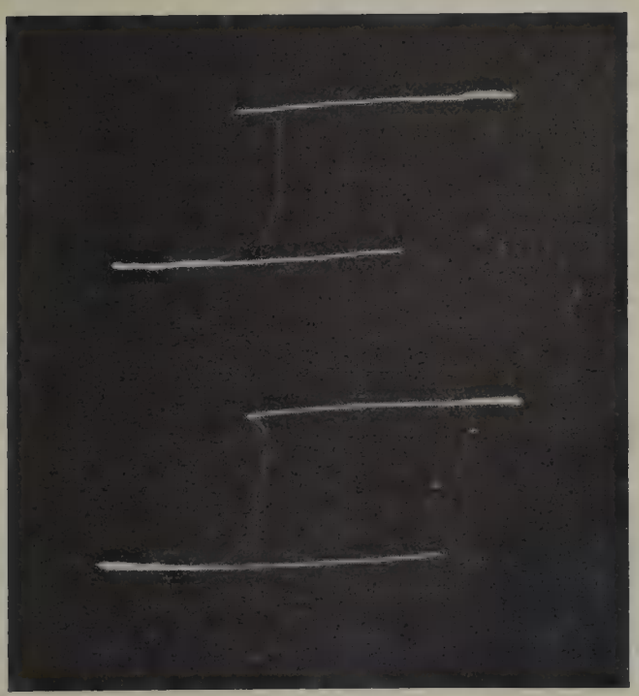

Fig. 1. Cobalt-ferrous ferrite. (*a*) Annealed with alternating field giving re-entrant loop; (*b*) annealed with unidirectional field giving loop re-entrant in one sense only. Applied 50 c/s field 35 Oe pk

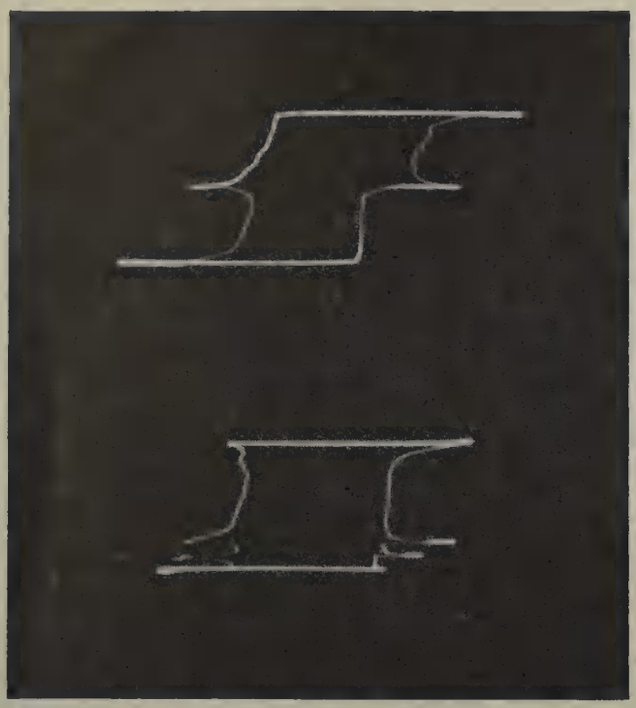

Fig. 2. Cobalt-ferrous ferrite. (*a*) Annealed in zero net remanence state giving tristable loop, and (*b*) annealed in intermediate remanence state. Applied 50 c/s field 17 Oe pk

By annealing a wall in an off-centre position, that is, with remanent magnetization between zero and full remanence, the relative position of the spikes on the two sides of the loop indicates whether the domain wall always moves in the same direction or oscillates to and fro. With the core geometry used (height greater than radial width) the spikes occur at the same flux-level (Fig. 2*b*) so that the wall evidently oscillates to and fro. Since cylindrical walls tend to move radially outwards, the field being strongest at the inner edge of the toroid, the movement is probably axial. Fig. 2*b* shows the loop resulting from the accidental annealing of two walls near one surface.

The same explanation holds for re-entrance; the wall is evidently not eliminated in the 'saturation' state and merely comes to rest near the sample surface. This is confirmed by the fact that excessive annealing fields which would tend to drive the wall out of the sample are found to reduce the re-entrance. The re-entrance of a partially annealed sample may be quite effectively increased by alternately switching from one remanent state to the other by the application of short pulses of alternate polarity (for example, 1-ms pulses with a pulse repetition frequency of 50 c/s). Thus annealing takes place substantially in the absence of an externally applied field. Further, when a unidirectional field is used for annealing, the loop is re-entrant on one side only (Fig. 1*b*). This would explain the asymmetry observed by previous workers using conventional display systems, for example, a ballistic galvanometer.

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A Numerical Model of Random Packing of Spheres

A numerical approach using a digital computer has been used to construct a simple model of the unit cell in a randomly packed bed of identical hard spheres. The cell is built up by introducing spheres tangent to a central one at random positions around it. Each added sphere is accepted and its position recorded only if it does not overlap with any of the ones previously accepted. When there is no room for additional spheres, the cluster is complete and the volume of the unit cell is found. This unit is defined as the polyhedron formed by the perpendicular bisectors of the lines connecting the centre of the original sphere with its neighbours. There is some ambiguity in defining a unit cell in a random packing. The prescription used here has the justification that it is correct for the regular lattices with one sphere per unit cell such as the simple cubic, face centred cubic, and hexagonal close-packed arrangements.

Number of neighbours and cell volumes. An ensemble of about 3,000 of the clusters already described here was constructed.

Table 1. Packing Statistics

n	Probability of n neighbours Touching	Probability of n neighbours Within 1·35 diam.	Average cell volume for n nearest neighbours
6	0·0054	0·0000	1·0349
7	0·0970	0·0026	0·9670
8	0·3878	0·1332	0·8924
9	0·3931	0·4316	0·8255
10	0·1137	0·3622	0·7688
11	0·0030	0·0638	0·7451
12	0·0000	0·0065	—
	$\bar{n} = 8{\cdot}52$	9·37	$\bar{v} = 0{\cdot}860$
	$\sqrt{\overline{n^2} - \bar{n}^2}$	0·84	$\sqrt{\overline{v^2} - \bar{v}^2}$

Table 1 gives the calculated frequency distributions of numbers of neighbours. In addition the average cell volume for each configuration is given, based on a unit ball diameter. Since just one ball is enclosed in the unit cell as defined here, the average solid fraction is given by:

$$f = (4/3)\pi(1/2)^3/0{\cdot}860 = 0{\cdot}609.$$

With the definition of the unit cell used here the proper solid fractions are obtained for the regular lattices as already mentioned.

Since packing densities between 0·61 and 0·64 have been found[1] depending on how well the bed of spheres has been shaken down, it appears that this model is describing a loose packing. This appears to be reasonable in view of the fact that no shaking down is allowed for in the process by which the ensemble of cells was constructed here.

In an experiment Bernal and Mason[2] found the mean number of contact points to be 8·5 counting only points in what they called total contact in a random close-packed assembly. Although this agrees superficially with the results obtained here, their distributions of numbers of contact points for both tight and loose packings were not the same as the present one. Fig. 1 shows the frequencies of various numbers of neighbours. Indeed, they observed cases with as few as four contact points. It is difficult to make a direct comparison with Bernal and Mason's results since their 'total contacts' include not only neighbours which touch the central ball but also neighbours somewhat farther out.

An attempt was made to include second neighbours in the cluster. This was done by filling in all the additional spheres that could rest without any overlapping on the stable positions determined by the nearest neighbours already found as described above. These positions were filled in random order.

Fig. 2 shows the density of ball centres as a function of distance from the centre of the cluster, both as found

here and as measured by Scott[3]. The peak describing the first neighbours determined in the present calculation, which should actually be a delta function of area 8·52, is broadened in the plot to match the class width used by Scott in his measurement. It is clear from the plot that the second neighbours obtained by the process described here do not exhaust all the possibilities of nearby spheres.

Angular distribution. The angular distribution of neighbours around any given neighbour taken as a pole is shown in Fig. 3. Distributions are given for neighbours within 1·35 ball diameters as calculated here and as measured by Scott and Mader[4]. There is qualitative agreement between calculation and measurement, but the detailed shapes are not the same.

Method of calculation. The process of building up the cluster by introducing spheres at random positions around the central sphere becomes very inefficient when the cluster is nearly complete. This is because the remaining regions where neighbours can fit into the cluster become extremely narrow when the cluster is nearly complete. To speed up the calculation the actual computational scheme was modified as follows. After 250 possible neighbours were examined it was ascertained analytically whether and where there were gaps into which additional spheres could be fitted as neighbours. Each such gap was filled by inserting a ball at the centre of the gap. This introduced no discernible error since after 250 tries the latitude in placement of any additional sphere or spheres is extremely small.

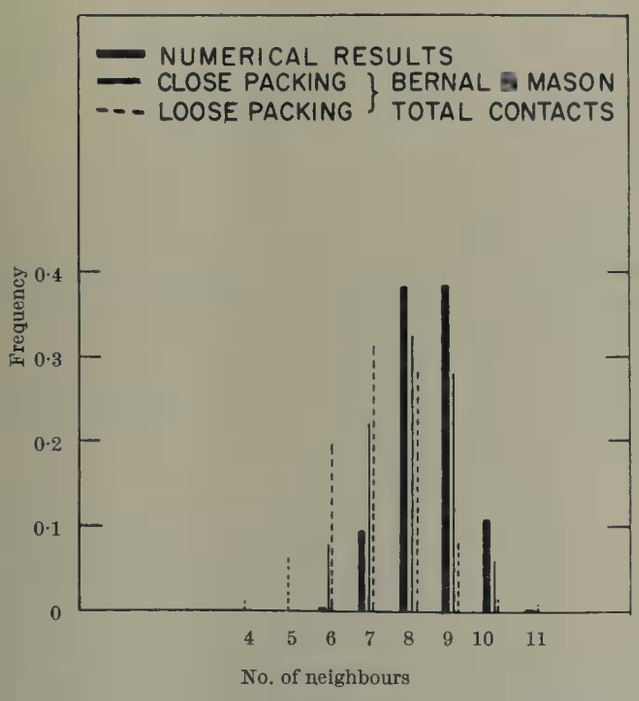

Fig. 1. Frequency distribution of nearest neighbours

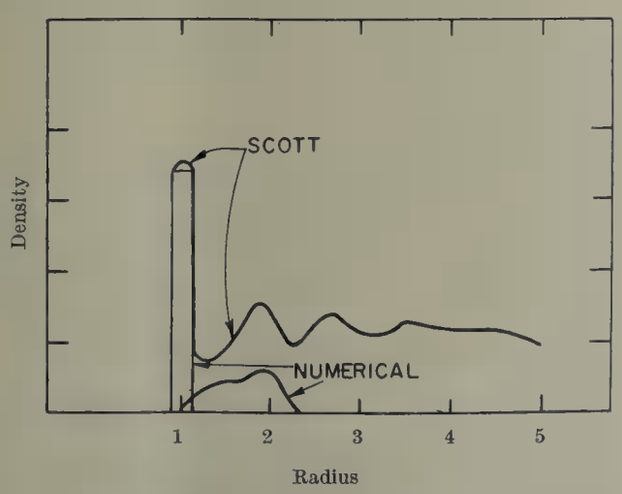

Fig. 2. Density of centres versus radial distance

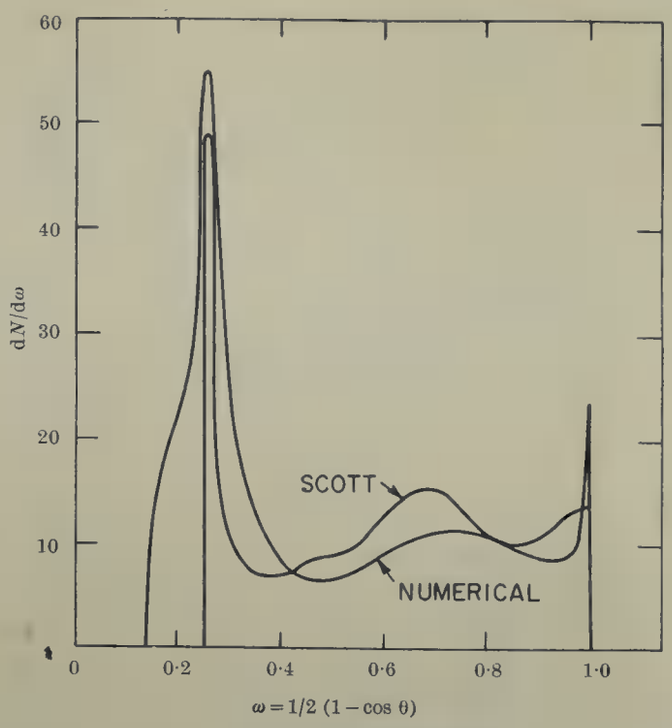

Fig. 3. Angular distribution of neighbours

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METALLURGY

Slow-bend Testing of Hydrided Zirconium

The embrittlement of zirconium by hydrogen is generally reflected by a decrease in the energy absorbed in fracture in an impact test, with a notched or unnotched specimen. Only in certain circumstances is a simultaneous reduction in ductility observed in a tensile test, and therefore the embrittlement has generally been considered attributable to high strain rates. However, some recent work we have carried out indicates that it is the actual mode of stressing—by bending—rather than the strain rate which promotes brittle fracture of hydrided zirconium in an impact test. Afterwards, slow-bend tests, both notched and unnotched, have proved useful in elucidating several of the factors which contribute to brittle fracture of zirconium. The relative unimportance of strain rate has been demonstrated by the occurrence of a brittle crack in a notched slow-bend test in which the bending to promote fracture took 48 h.

The slow-bend test also readily distinguishes between low-energy ductile failures and brittle cracks whereas the normal impact test does not. The three basic types of deformation curve obtained in slow-bend testing of hydrided zirconium are shown in Fig. 1, the deformation being taken simply as the movement of the straining head on the machine. The completely brittle specimen is typified by *A* which shows an elastic region I, and a plastic region II which varies slightly in extent. Once started, the crack is self-propagating, at a speed in the region of 25 m/sec, and needs no further deformation.

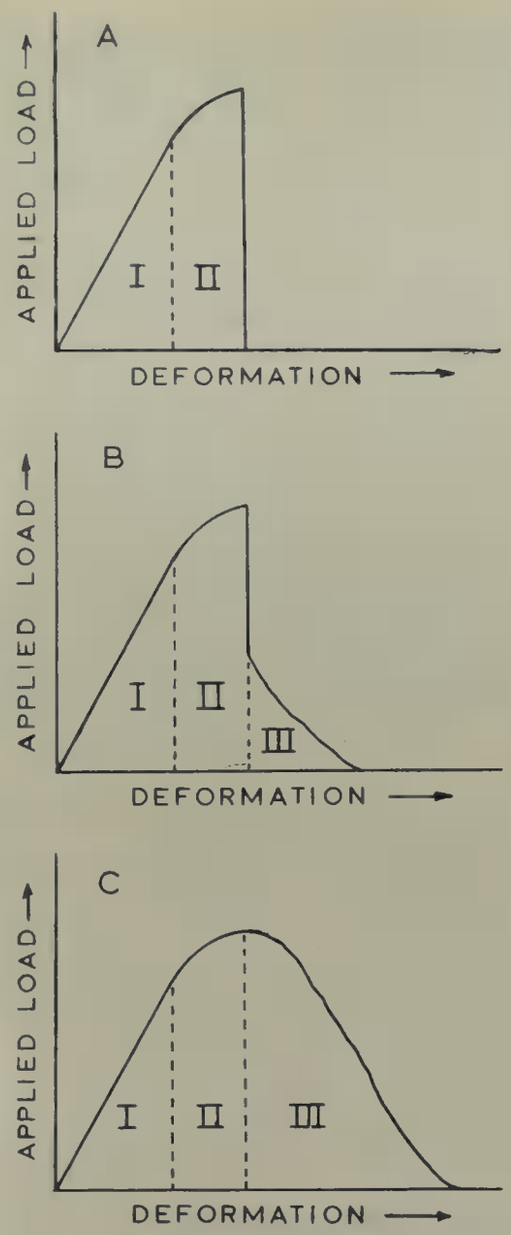

Fig. 1. Typical load-deformation curves for slow-bend tests on hydrided zirconium. *A*, Brittle fracture; *B*, low-energy ductile failure; *C*, high-energy ductile failure

On the other hand, a ductile specimen, as typified by *C*, requires continuous application of load throughout to promote propagation of a crack through the specimen, and therefore the rate of propagation is dependent on the rate of application of load. An additional region III in the curve represents the energy required for propagation.

Certain specimens give an intermediate type of curve, such as *B*, but the actual cracking is still ductile and requires a certain amount of further bending to cause propagation, but is preceded by a 'yield drop', during which 'ears' of plastic flow suddenly extend right across the sides of the specimen from the root of the notch, which has the effect of reducing the area of region III. Since the area under the deformation curve represents the energy required to fracture the specimen, as measured in a standard impact test, the usual impact test would not readily distinguish between a brittle specimen and a ductile one having a deformation curve of type *B* with an appreciable 'yield drop'.

It is interesting to note that the general shape of the load-deformation curves is similar to that of the load-time curves dynamically recorded by Fearnehough and Hoy[1] during the Charpy impact testing of steels. Load-deformation curves for slow-bend tests are, however, much more readily obtained than load-time curves in a high-speed test.

Using a standard Hounsfield slow-bend specimen in the usual slow-bend jig, but attaching the latter to a hard-beam testing machine, the effect of various variables on the hydrogen embrittlement of zirconium has been studied more effectively than could be accomplished by standard impact tests. The results indicate the importance of the 'yield drop' and its dependence on temperature, hydrogen content and inter-hydride spacing. The latter is a very important factor which may vary considerably with grain size and heat treatment even with a constant hydrogen content.

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MINERALOGY

Structure of Interstratified (Mixed-layer) Minerals

Many papers dealing with the structure of interstratified minerals such as illite-montmorillonite, chlorite-montmorillonite, chlorite-vermiculite, etc., have been published up to the present. In describing the structure of these minerals, the terms 'random' and 'regular' have been often used, but their definition is not very clear, since the term 'regular' has been applied mostly to '1:1 regular structure', whereas the term 'random' has been used for structures other than regular. It is necessary to investigate in detail the structure of interstratified minerals.

Suppose two kinds of layers, A and B, their number, N_A and N_B ($N_A + N_B = N$), and the potential energy between two layers, $-\varphi_{AA}$, $-\varphi_{AB}$, $-\varphi_{BA}$, $-\varphi_{BB}$, assuming the nearest-neighbour interactions, that are equivalent to Reichweite $g = 1$. Then the total interaction energy (E) in a one-dimensional polar structure can be expressed by statistical mechanics as follows:

$$E = -\varphi_{AA}N_A - \varphi_{BB}N_B + \{\varphi_{AA} + \varphi_{BB} - (\varphi_{AB} + \varphi_{BA})\} \cdot N_{AB}$$

N_{AB}: the number of A-B pairs.

In the foregoing expression, $\varphi_{AA} + \varphi_{BB} < \varphi_{AB} + \varphi_{BA}$ is a condition necessary for the formation of the interstratified structure. For definite values of N_A and N_B, E is determined by the value of N_{AB}. This suggests that the stability of the structure increases energetically in proportion to the number of A-B pairs. We define here W_S as the probability of finding S layer at any position and P_{ST} as the probability of finding T layer succeeding S layer.

Then N_{AB} is shown as $N_{AB} = W_{AB} \cdot N = W_A \cdot P_{AB} \cdot N$, where W_{AB} is the probability of finding an A-B pair at any position.

Thus we can calculate the maximum value of W_{AB} for two cases, $g = 0$ and $g = 1$.

(*a*) For $g = 0$ (random), which is a special case of $g = 1$, the following relation holds: $P_{AB} = P_{BB} = W_B$, $P_{BA} = P_{AA} = W_A$.

In this case, W_{AB} equals $W_A \cdot W_B$, and this is the only one result given for $g = 0$.

(*b*) For $g = 1$, the maximum value of W_{AB} can be mathematically shown as follows:

(1) $W_A = W_B$ $\qquad W_{AB} = 2\ W_A \cdot W_B > W_A \cdot W_B\ (g = 0)$

(2) $W_A \neq W_B\ (W_A > W_B)$ $\qquad W_{AB} = W_B > W_A \cdot W_B$

In case (1), the relation of $P_{AA} = P_{BB} = 0$, $P_{BA} = P_{AB} = 1$ is held. This implies a 1:1 regular interstratified structure, $ABABABAB\ldots$. In case (2), the relation

of $P_{BB}=0$, $P_{BA}=1$ is held. This implies a structure having no B-B pairs, being neither 'regular' nor 'random'. In this case two arrangements, $ABAAABA$ and $ABAABAA$, are equivalent in the total interaction energy, having equal numbers of A-B pairs, so it is expected that all such possible arrangements would occur with equal probability for $g=1$.

Next we put $P_{AA}=\alpha$, $P_{AB}=1-\alpha$, $P_{BB}=\beta$, $P_{BA}=1-\beta$, then an equation $\beta=K\alpha+(1-K)$, where $K=W_A/W_B$, can be derived from the relation $\sum_S W_S P_{ST}=W_T$. Using this equation all the interstratified structures consisting of two kinds of layers can be plotted on the graph shown in Fig. 1.

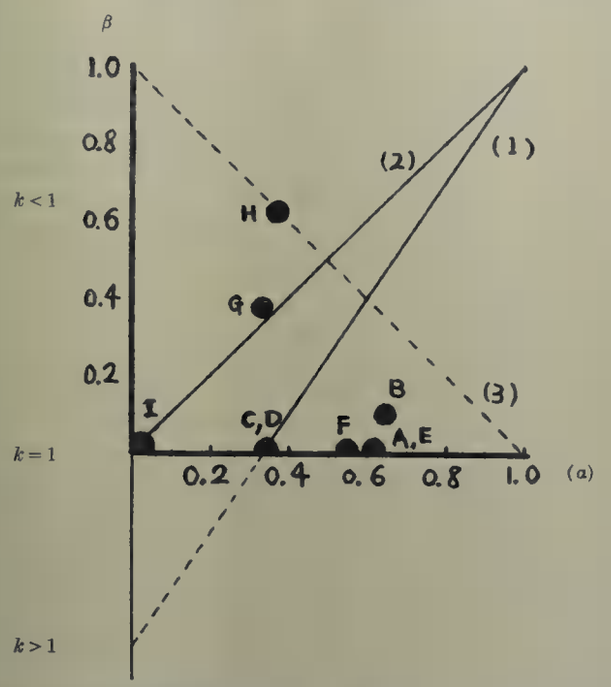

Fig. 1. The interstratified structure and some examples of illite-montmorillonite interstratified minerals examined

For example, the structure consisting of $W_A=0{\cdot}6$, $W_B=0{\cdot}4$, that is, $K=3/2$, is plotted on line (1), and $W_A=0{\cdot}5$, $W_B=0{\cdot}5$, on line (2). The random structures are on the diagonal dotted line (3). From the above view of interaction energy, it is expected that many interstratified minerals are distributed along or near the axes of co-ordinates.

In order to confirm this hypothesis, it is necessary to determine the values of W_A, W_B, α and β in a given specimen. Observed (00ζ) reflexions of X-rays, where ζ is not an integer, were compared with mathematical intensity distribution due to one-dimensionally disordered structure. The equation used, which was presented by Kakinoki and Komura[1,2], is as follows:

$$I(\xi\eta\zeta)=N\,\bar{V}^2+\sum_{m=1}^{N-1}(N-m)\,\mathrm{Spur}\,\mathbf{V}\,\mathbf{F}\,\mathbf{Q}^m+\mathrm{conj.}$$

$\mathbf{V}$ is a matrix of layer-form factors V_S, $\mathbf{F}$ is a matrix of W_S, $\mathbf{Q}$ a matrix of P_{ST} multiplied by phase shift. N is the total number of layers. I programmed the equation for an electronic computer 'HIPAC 103', and the calculation for various combinations of W_A, W_B, α and β of illite-montmorillonite, chlorite-montmorillonite, etc., is now in progress. Results of some of the illite-montmorillonite interstratified minerals examined are plotted in Fig. 1.

(A) and (B) are from Kamisunagawa in Hokkaido[3], (C) Honami mine in Nagano Pref.[4], and (D) Kamikita mine in Aomori Pref.

(E), (F), (G) and (H) are the specimens investigated by MacEwan[5] using the Fourier transform method. (I) is the 1:1 regular structures reported by other investigators[4,6,7]. The result seems to support the foregoing view, and suggests that it is necessary to treat the interstratified structures for $g\geqslant 1$, not $g=0$ (random).

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CHEMISTRY

Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography

It is known that the optical isomers of many amino-acids can be metabolized by different pathways although it is often difficult to obtain suitable preparations for investigation. Several methods, both biological and chemical, are available for obtaining individual isomers in a pure state[1,2], but many of these tend to be practicable only if a large amount of the racemic form is available; yields obtained by classical chemical separation methods are low, while biological procedures usually lead to the destruction of one of the isomers. Separation has been achieved, however, by paper chromatography[3–6]. This approach seemed to be best suited to our requirement, the isolation of pure optical isomers of ^{14}C-5-hydroxy-DL-tryptophan, a comparatively expensive compound. During our investigation, we noted that 5-hydroxy-DL-tryptophan (DL-5HTP), 6-hydroxy-DL-tryptophan (DL-6HTP) and DL-tryptophan (DL-TP) separated into two diffuse but distinct spots when chromatographed on Whatman No. 1 paper with butanol–pyridine–water (1 : 1 : 1, by vol.) as solvent system. The possibility of improving this separation by thin-layer chromatography was therefore explored.

Thin-layer plates (20 cm × 20 cm) were coated with a mixture obtained by homogenizing cellulose powder *CC*41 (Whatman, Ltd.) and water (1 : 2·3, w/v) for 30 sec at maximum speed in an M.S.E. blade homogenizer. A thin layer, 0·3 or 0·4 mm in thickness, was obtained by using a Camag applicator. Plates were air-dried overnight at room temperature, or oven-dried at 105° for 10 min. The compounds, dissolved in methanol or water, were applied to the thin layer and developed in the same solvent system as that used for paper chromatography. The solvent front was allowed to run for a distance of 10 cm which normally took about 1 h at room temperature. Plates were dried in a current of air at room temperature and sprayed with Ehrlich's reagent. The lowest concentration of DL-5HTP detected by this reagent was 0·05–0·1 μg. Table 1 shows the separation of isomers of the three amino-acids obtained by this technique.

It will be seen that the D-isomers of all three compounds have a greater R_F value than the corresponding L-isomers. D-5HTP used as a chromatographic marker was obtained by the action of L-amino-acid oxidase on DL-5HTP (ref. 7). L-5HTP was identified by incubation with guinea-pig kidney L-aromatic amino-acid decarboxylase[8], followed by chromatography of the reaction products, which showed a disappearance of the L-5HTP and the

Table 1. R_F Values of the Optical Isomers of DL-TP, DL-5HTP, and DL-6HTP chromatographed on Cellulose Thin Layer in Butanol–Pyridine–Water (1 : 1 : 1) Solvent System

	DL-TP	DL-5HTP	DL-6HTP
L-Isomer	0·52	0·47	0·36
D-Isomer	0·62	0·54	0·41

appearance of another spot, identified as 5-hydroxytryptamine. 6HTP was obtained presumably in the L-form from the hydroxylation of L-TP by *Chromobacterium violaceum*[9].

To separate the isomers of DL-5HTP on a preparative scale, the compound was dissolved in methanol–0·1 N hydrochloric acid (5 : 1, by vol.) at a concentration of 25 mg/ml. and applied as a fine streak, 15 cm long, to a thin-layer plate. Using an applicator[10], it is possible to apply several streaks on the same position after drying between each application. The application of two streaks was generally convenient, as this allowed the separation of about 1 mg of DL-5HTP. The chromatogram was developed in the solvent and, after drying, was sprayed with a 0·1 N solution of methanolic hydrochloric acid. The plate was viewed under ultra-violet light at 254 mμ (Camag 'Universal UV' lamp) and the outline of the two isomers delineated by their pink fluorescence. The *CC*41 powder corresponding to these areas was sucked into two tubes, each representing a different isomer. The powder was extracted with methanol and extracts were re-streaked and run on separate chromatograms. On viewing the two chromatograms under ultra-violet light, any contaminating isomer was seen to be well separated. Isomers were extracted with methanol as before and chromatographed, when each ran as a single spot.

When up to 1 mg of DL-5HTP is chromatographed, we have found that there is a certain amount of overlapping of the two isomers. By selecting the area of each isomer in such a way as to exclude this area of overlap, it is possible to obtain a fairly pure sample of each isomer in approximately 50 per cent yield. The area containing the mixed isomers could be kept separate and pooled with similar areas of other chromatograms. The pooled isomers could then be re-chromatographed to increase the overall recovery of the individual isomers.

Equilibration of the chromatogram for periods of up to one hour did not result in a better separation of the isomers; separation is, however, slightly improved if chromatography is carried out at 4°; this is presumably due to the increased developing time of the chromatogram.

We now hope to use this technique for separating the D- and L-isomers of ^{14}C-5-hydroxy-DL-tryptophan for metabolic investigations.

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Mechanism of Radical Decay in Irradiated Polyethylene

Investigations of the decay of free radicals in irradiated polyethylene demonstrate that the sec-alkyl radicals, formed with a *G*-value of about 3, decay at a significant rate at room temperature to leave a much smaller number of allyl-type radicals that decay at a very much slower rate.

Although there is no direct proof that the cross-linking process involves combination of these free radicals, the correspondence in *G*-value between radical formation and cross-linking, and the fact that cross-linking results from ultra-violet irradiation in the presence of sensitizers[1], constitute strong evidence that cross-linking is a radical combination process.

There has been much discussion in the literature as to whether the alkyl radicals decay by first-order or second-order kinetics. Lawton, Balwit and Powell[2] believed the decay to fit four first-order reactions with different decay constants. Cracco, Arvia and Dole[3] fit their data with two first-order decay constants. Charlesby, Libby and Ormerod[5], and Ormerod[6], found their data to be consistent with a single decay constant of second order.

There is fairly general agreement that a very fast decay occurs in the initial stages, caused by a non-random distribution of radicals occasioned by their presence as near-neighbours. Since the initial deposition of energy in spurs would produce this distribution, it is not really necessary for this argument to postulate invariable production of radicals as near-neighbour pairs[2].

Since radical decay occurs even in the crystal[7], where physical diffusion of the polymeric radicals and even significant segmental motion are prevented, it is necessary to postulate that either radicals are produced in very close proximity, or there is a chemical mode of radical migration. If the radicals are in groups in very close proximity, rate of decay should be first order, but there should be a continuous gradation of first-order rate constants, corresponding to near neighbours, more distant neighbours, and still more distant neighbour pairs reacting by limited segmental motion. If the radicals are not all in such close groups, a chemical mechanism for mobility must be present and the reaction will be second order after the initial combination of nearest neighbours.

Dole, Keeling and Rose[8] first proposed that radical sites could migrate by what might be formulated as a succession of radical abstraction reactions:

$$R + R'H \rightarrow RH + R'$$

in which the alkyl radicals are of the secondary type. Koritskii *et al.*[9] supported this hypothesis. It is of interest to see whether known reaction rates for this reaction could provide a free radical diffusion constant that would give a second-order rate constant for radical decay that is of the right magnitude.

With each abstraction reaction the radical site moves an estimated 2·3 Å (average distance between hydrogens in the system). According to three-dimensional random walk theory, a radical which makes n jumps each of length l in random directions will travel a mean distance r from its starting point, given by:

$$r^2 = l^2 n$$

In particle theory the relationship of the diffusion constant to this mean square distance per unit time is given by:

$$D = \frac{1}{6}\frac{dr^2}{dt}$$

and we see no reason that this cannot be applied here as representing the constant for diffusion of the radical sites.

The relationship of a second-order rate constant to the diffusion constants of the reactants in a diffusion-controlled reaction is given by:

$$K = 4\pi r_0(D_1 + D_2)$$

where r_0 is the distance at which reaction will occur. In this instance, K is the diffusion-controlled rate constant for radical combination in this system.

From these expressions:

$$K = \frac{4\pi}{3} r_0 l^2 \frac{dn}{dt}$$

The reaction frequency, dn/dt, is the number of hydrogen abstractions per second undergone by each radical. This equals $K_a C_H$, where K_a is the rate constant for the abstraction reaction and C_H is the concentration of hydrogen atoms attached to secondary carbon atoms. The latter is equal to l^{-3}. We also assume r_0 is also approximately equal to l, the average distance between hydrogen atoms. From this:

$$K = \frac{4}{3}\pi K_a = 4{\cdot}2\,K_a$$

The diffusion-controlled rate constant for radical combination K can be calculated if we can determine the value of K_a. The rate constant K_a is not known for condensed phase and large molecules, and can only be estimated indirectly.

For gas phase, James and Steacie[10] have determined the rate constant for abstractions of secondary hydrogens from *n*-heptane by ethyl radicals. If we consider the secondary hydrogens as one reactant, the rate constant at 25° is 120×10^{-23} c.c. molecule^{-1} sec^{-1}.

No direct data are available on rate constants for such abstraction by secondary alkyl radicals. However, Kerr and Trotman-Dickenson[11] have tabulated such data for abstractions from aldehydes, and show that the rate constants for abstraction by isopropyl and *n*-propyl radicals are very nearly the same. Hoey and LeRoy[12] and Wijnen and Steacie[13] provide data from which it can be concluded that the ratio of rate constants for the reactions $i\mathrm{Pr}\cdot + \mathrm{H_2} \rightarrow i\mathrm{PrH} + \mathrm{H}\cdot$ and $\mathrm{Et} + \mathrm{H_2} \rightarrow \mathrm{EtH} + \mathrm{H}\cdot$ is about 0·2. On this basis we estimate the rate constant for abstraction of secondary hydrogens by secondary alkyl radicals to be roughly 40×10^{-23} c.c. molecule^{-1} sec^{-1}.

If we can assume that this rate constant is applicable to segments of the polymer molecule in the amorphous phase above the glass transition temperature, we have as a diffusion-controlled rate constant for radical combination at 25°:

$$K = 4{\cdot}2\,K_a = 170 \times 10^{-23} \text{ c.c. radical}^{-1} \text{ sec}^{-1}$$

The second-order rate constants for free radical disappearance in irradiated polyethylene measured by Charlesby *et al.*[5] have values ranging from 1×10^{-23} c.c. radical^{-1} sec^{-1} to 200×10^{-23} c.c. radical^{-1} sec^{-1}. The higher value, obtained with high-pressure polyethylene, is in reasonable agreement with that calculated above. The low values were obtained with high-density, rather crystalline polyethylene. For these, the chains are held relatively immobile, and the gas-phase rate constant would be inapplicable on both steric and energetic grounds.

While the foregoing considerations seem of value for the bulk of the decay, the importance of the initial spur reaction must still be considered. The question involves the fraction of radicals that must react before the remainder can be assumed to have random distribution. Ohnishi's[14] results have special significance here. He found that irradiation of linear polyethylene at −196° gave a steady-state radical concentration of $2{\cdot}6 \times 10^{-4}$ moles/g. At this concentration, the average separation of radicals is 18 Å, or, assuming grouping of four to a spur, the average separation of spurs is 30 Å. With this spacing, added ionization and excitation destroys as many radicals as it creates. Thus, at 50 Mrads, at which 90 per cent of the steady-state value is reached, spur overlap is already very serious, and the validity of any influence of non-random distribution is questionable at such high doses.

There has been much speculation about the role of hydrogen in enhancing mobility by the reactions:

$$R\cdot + \mathrm{H_2} \rightarrow R\mathrm{H} + \mathrm{H}\cdot$$
$$\mathrm{H}\cdot + R\mathrm{H} \rightarrow \mathrm{H_2} + R\cdot$$

Dole and Cracco[15] demonstrated its occurrence at a rate paralleling the rate of decay of radicals; they found the exchange ceased when the alkyl radicals had disappeared. However, they calculated that an average of only one or two exchanges of this type occurred per alkyl radical. It is of interest to calculate the rate expected from gas-phase rate constants.

Hoey and LeRoy[12] determined the activation energy for the reaction:

$$i\mathrm{Pr}\cdot + \mathrm{H_2} \rightarrow \mathrm{C_3H_8} + \mathrm{H}\cdot$$

to be 12·5 kcal/mole. This is 1·2 kcal/mole larger than the similar quantity for the reaction of ethyl radical. Wijnen and Steacie[13] determined the activation energy for ethyl + deuterium to be 13·3 kcal. If we, therefore, take 14·5 kcal/mole for the activation energy for the reaction:

$$i\mathrm{Pr}\cdot + \mathrm{D_2} \rightarrow \mathrm{C_3H_7D} + \mathrm{D}\cdot$$

and a steric factor of 10^{-3}, we obtain a rate constant at room temperature of about $1{\cdot}3 \times 10^{-23}$ c.c. molecule^{-1} sec^{-1}. In the system of Dole and Cracco, assuming radicals in chain segments have a similar rate constant for reaction with D_2 and using their value for the concentration of deuterium in the solid, we calculate the frequency of reaction of a radical with D_2 should be:

$$1{\cdot}3(10^{-23})\,(C_{\mathrm{D_2}}) = 1{\cdot}3(10^{-23})\,(1{\cdot}2)\,(10^{-17}) = 1{\cdot}6 \times 10^{-6} \text{ reactions/sec}$$

and a radical should react on the average once in 170 h. This is about a factor of ten longer than observed; but the agreement is reasonable for the approximations involved.

This reaction is about a million times slower than the abstraction from alkane, which occurs about thirty times per second with each radical. Thus, it is difficult to understand how the presence of hydrogen enhances radical combination by a significant extent. Yet Smith and Jacobs[4], Cracco, Arvia and Dole[3], and especially Ormerod[6], noted that presence of an atmosphere of hydrogen enhances the rate constant for radical combination by a factor of about ten. Thus, there is still no adequate explanation for this effect.

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Irradiation Embrittlement of Low-boron Type 304 Stainless Steel

There is a growing amount of literature[1–7] that describes the magnitude and characteristics of the irradiation-induced embrittlement of alloys at high temperatures. These data appear to be consistent with the hypothesis that the grain boundary embrittlement is due to the helium generated by the ^{10}B (n,α) (ref. 7) Li thermal neutron capture reaction[1,8,9].

This communication describes the effect of very small concentrations of boron on the irradiation embrittlement of 'type 304' stainless steel irradiated to a high exposure in a thermal neutron test reactor. These steels generally contain more than 1 p.p.m. B and it was necessary to electron-beam re-melt a commercial stainless steel to reduce the concentration of this element to very low values.

Five charges of approximately 650 g were prepared by cutting 1/4-in.-diameter rod into 2-in. lengths. The charges were melted using an 18-kW power-level and melting times of 4, 8, 12, 16 and 20 min. The resulting 'pancakes' were 4 in. in diameter and 7/16 in. thick. The chemistry of these pancakes is representative of the austenitic stainless steel class.

The boron concentration was determined using the spark source mass spectrograph of the Mattauch-Herzog geometry, which utilizes a radio frequency spark to produce ionization of the sample. The resulting ions are focused according to energy and direction and collected on an Ilford '*Q* II' photographic plate. Elements are identified by position on the plate and concentrations are estimated from the density of the line as compared with an internal standard. In all samples of this series ^{53}Cr was used as an internal standard. Chromium-53 is a minor isotope and was free of any interference from other constituents of the sample. The samples were sparked so that a maximum exposure of 0·3 microcoulomb was obtained. For samples suspected, or known, to have high boron content, a 0·1-microcoulomb exposure was obtained. Detection limit varied from 5×10^{-9} to 8×10^{-8} atom fraction depending on exposure, plate and sample. In no case was the boron concentration lower than the detection limit for that sample. Results are summarized in Table 1. These data are believed to be accurate to within ± 50 per cent.

Table 1. Boron Concentration by Weight

Alloy	Re-melting time in furnace (min)	Natural boron (p.p.m.)	Detection limit (p.p.m.)
18141*BC*	0	3·90	0·016
*EBM*706	8	0·130	0·003
*EBM*707	12	0·015	0·003
*EBM*709	16	0·107	0·003
*EBM*710	20	0·023	0·001

After the various heats had been analysed, the electron-beam melted alloys and the commercial heat (in the as-received condition) were machined into subsize tensile specimens which were then irradiated in the core of the Oak Ridge Research Reactor at a temperature of 700° C. The samples were then removed from the reactor and tension-tested in a hot cell using an Instron machine. The post-irradiation ductilities, as given by the true uniform strain and total elongation, are listed in Table 2 for deformation at 704° and 842° C.

Table 2. Embrittlement of Stainless Steel irradiated at 700° C

Tensile test temperature (° C)	Natural boron concentration (p.p.m.)	Ductility* True uniform strain† Irradiated (%)	Unirradiated (%)	Total elongation Irradiated (%)	Unirradiated (%)
704	0·015	13·7		19·1	
	0·023	12·7	30·9	23·3	56·2
	0·107	13·1		21·6	
	0·130	12·7	19·6	19·6	60·4
	3·9	11·2	18	13·1	40·2
842	0·015	3·1		6·1	
	0·023	2·9		6·1	
	0·107	2·9	13·8	5·6	46·6
	0·130	3·3	18·2	6·1	57·2
	3·9	1·5	16·0	3·0	43·0

* Irradiated at B-8 position of the Oak Ridge Research Reactor to a neutron dose of $4{\cdot}5 \times 10^{20}$ neutrons/cm² (thermal) and $3{\cdot}5 \times 10^{20}$ neutrons/cm² (E > 1 MeV).
† Specimens strained at a rate of 0·2 per cent/min.

Only small variations are found in the ductilities of all the electron-beam melted heats, although an order of magnitude exists between the concentrations of boron. The largest difference in ductility exists between the commercial alloy and the electron-beam re-melted alloys. The concentrations of helium were calculated for the ^{10}B reaction for each of the alloys and are given in Table 3. If one considers only the (n,α) reactions due to thermal neutrons, these data suggest that helium is not responsible for the embrittlement or that the embrittlement is quite independent of helium concentrations in the range of $0{\cdot}13 - 1{\cdot}1 \times 10^{-7}$ atom fraction. However, if one computes the concentration of helium due to fast neutron (n,α) reactions for all the elements present in stainless steel, then these data appear more compatible

Table 3. Approximate Helium Concentration from All (n,α) Reactions

Alloy No.	Natural boron concentration (p.p.m.)	Helium concentration (atom fraction) Due to ^{10}B and thermal neutrons $\times 10^{-6}$	Due to fast neutron (n,α) reactions $\times 10^{-6}$	Total $\times 10^{-6}$
*EBM*707	0·015	0·013	0·3	0·313
*EBM*710	0·023	0·020	0·3	0·320
*EBM*709	0·107	0·091	0·3	0·391
*EBM*706	0·130	0·110	0·3	0·410
18141*BC*	3·9	3·3	0·3	3·6

with an irradiation embrittlement mechanism based on helium. The concentration of helium due to the (n,α) reactions with fast neutrons is also given in Table 3; these data were taken from the work of Bush, Moteff and Weir[10]. Considering the helium from all sources, the lack of variation in ductility of the electron-beam melted heats is more easily explained since the difference in total helium concentration is less than a factor of 2. This suggests that the irradiation of these electron-beam melted alloys in a more thermal neutron environment or to a lower fast neutron dose would result in a greater variation in the ductilities.

It is concluded that removing the ^{10}B from the alloy is not the ultimate solution for improving the ductility of irradiated alloys because, even in thermal neutron reactors, (n,α) reactions with fast neutrons are important, particularly for materials containing low boron.

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Boron–Nitrogen Bond Strengths in Borazoles

The bond order of boron–nitrogen compounds and the aromatic character of borazoles have been subjects of interest for many years (see, for example, ref. 1). It is shown here that bond energy terms and bond length measurements give apparently conflicting information about the relative strengths of some boron–nitrogen bonds.

The heats of hydrolysis in water at 25° C of *N*-trimethylborazole, borazole and *B*-trichloroborazole are − 0·7 ± 0·1, − 19·7 ± 0·3, and − 113·6 ± 0·2 kcal/mole respectively. The value for *B*-trichloroborazole agrees with the previously reported[2] value of − 113·8 ± 0·7 kcal/mole. The standard heats of formation of the compounds are − 232·3 ± 0·1, − 204·9 ± 0·3, and − 256·1 ± 0·2 kcal/mole respectively. This value for borazole differs significantly from − 131·1 ± 3·2 kcal/

Table 1. Boron–Nitrogen Bonds

Compound	E (kcal/mole)	r (Å)	Ref.
$B_3H_2N_3Me_3$	121·4	1·42 ± 0·02*	6
$B_3H_3N_3H_3$	117·0	1·47 ± 0·07*	7
		1·44 ± 0·02*	8
$B_3Cl_3N_3H_3$	106·5‡	1·41 ± 0·02*	6
		1·413 ± 0·01†	9
		1·415†	10

* Electron diffraction.
† X-ray diffraction.
‡ Compare with ref. 5.

mole deduced from the heat of combustion[3]. The boron–nitrogen bond energy terms, calculated from the standard heats of formation, the heats of vaporization or sublimation[4], the heats of atomization of the elements[5], and bond energy terms characteristic of other bonds in the molecules[5], decrease in the order:

$$B_3H_3N_3Me_3 > B_3H_3N_3H_3 > B_3Cl_3N_3H_3.$$

Bond energy terms, E, and internuclear distances, r, of the boron–nitrogen bonds in the three compounds are compared in Table 1. The differences between the bond length measurements are small compared with the experimental errors, but both series of measurements indicate that the boron–nitrogen bond order is greater in *N*-trimethylborazole than in borazole. The thermochemical data suggest that the bond order is least in the case of *B*-trichloroborazole. This is in qualitative agreement with the original interpretation of the ultra-violet spectra[11] and with the relative values of the diamagnetic anisotropy of borazole and *B*-trichloroborazole[12].

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Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography

The highly specific response of the Lovelock electron capture detector toward certain functional groups has been exploited in various ways, notably in the detection of trace amounts of halogenated compounds[1].

In this laboratory the electron capture detector has been found to be extremely sensitive to carbon disulphide, and the presence of low concentrations of this compound is readily detected in hydrocarbon solvents. In the case of benzene, the S.T.P.T.C. standard colorimetric method[2] already exists, but the use of electron capture gas-liquid chromatography would appear to offer the advantages of increased analysis speed and improved accuracy.

A 10-ft. gas-liquid chromatography column is used to separate the CS_2 from other compounds. This is packed with 5 per cent w/w trixylenyl phosphate on 60/72 B.S.S. 'Celite' and operated at 70° C with nitrogen as carrier gas. The electron capture detector is of conventional construction with an internal volume of 1 ml. Tritium is used in the cell as a source of electrons and the cell is operated at a constant potential difference of 60 volts. A conventional electrometer amplifier is connected to the detector and the output is measured on a 1-mV high-speed recorder after appropriate attenuation. A typical chromatogram of a 10-μl. sample of benzene containing 1·0 p.p.m. by weight sulphur as CS_2 is shown in Fig. 1.

Fig. 2 shows the relationship between CS_2 peak height and concentration. The graphs were obtained from standard solutions of CS_2 in benzene prepared by the S.T.P.T.C. procedure (ref. 2). A sample volume of 10 μl. was used for the range 0·1–1·0 p.p.m. and a volume of 1·0 μl. for the range 1·0–10·0 p.p.m., precision microsyringes being used in each case.

The results are linear over both ranges, although the lower concentration range shows a slight deviation from the origin. The lowest concentration of CS_2 used in the calibration, namely 0·1 p.p.m., corresponds to a peak height of 0·5 cm and this was clearly seen against the background noise. The minimum limit detectable would be about 0·05 p.p.m. which would correspond to a signal-to-noise ratio of 2:1.

Parallel calibrations were also made of the S.T.P.T.C. standard method. This involves measuring the optical

Fig. 1. Electron capture chromatogram of benzene containing 1·0 p.p.m. CS_2. *a*, Paraffins; *b*, CS_2; *c*, *cyclo*hexane; *d*, benzene; *e*, toluene

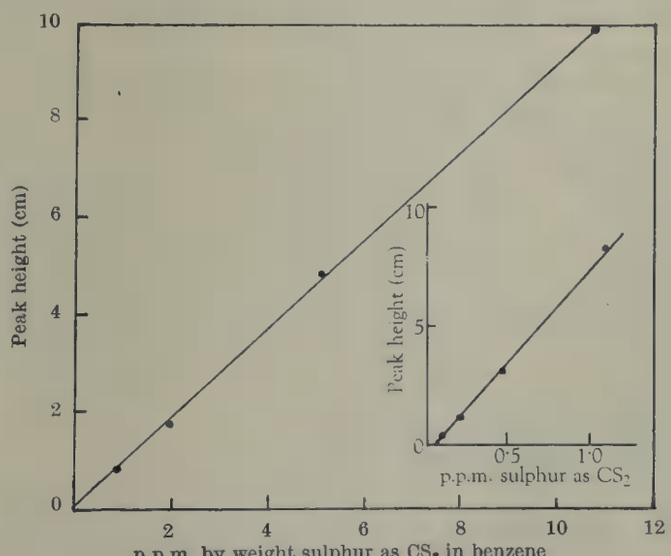

Fig. 2. Relationship between CS_2 peak height and electron capture response

density of the colour formed, after the addition of copper acetate and diethylamine, by means of a photoelectric absorptiometer. The graphs showed considerable deviations from linearity outside the range 0·5–5·0 p.p.m. CS_2.

Table 1 shows a comparison of the two methods when used for the analysis of three benzene samples.

Table 1. Sulphur as Carbon Disulphide (p.p.m. by weight)

	S.T.P.T.C. standard method	G.L.C. method
Sample 1	6·3	6·4
Sample 2	0·4	< 0·1
Sample 3	0·3	< 0·1

A good agreement is obtained in the case of sample 1 having a relatively high CS_2 content.

The gas-liquid chromatography (G.L.C.) method is probably more accurate than the S.T.P.T.C. method with samples 2 and 3, since these results were based on the absence of any CS_2 peak, in contrast with a calibration sample containing 0·1 p.p.m. of this compound.

Table 2

Injection number	1	2	3	4	5	6	7	8	9	10
Peak ht. (cm)	10·50	10·76	11·21	10·95	10·65	10·95	10·75	11·15	11·35	11·15

Mean peak height = 10·94 cm
Standard deviation = 0·274 cm
Per cent standard deviation = 2·5

Table 2 gives the results of ten repeat injections of a 10-μl. sample of benzene containing 1·0 p.p.m. sulphur as CS_2.

The results indicate a relatively high degree of repeatability for this type of analysis.

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Separation of Coumarins

By sorption on ion exchange resins[1–3] it is possible to separate some coumarins in dilute solution. A column of the strongly basic anion exchange resin, 'Amberlite *IRA*–400' (−40, +60 mesh; Rohm and Haas Co.), in the chloride form (bed length, 50 cm; bed volume, 25·8 c.c.) has been used for this purpose.

The coumarins studied were of three groups. Group *A* includes coumarin (I), 3-Me(I), 7-Me(I), 3,4-di Me(I), 6,7-di MeO-4-Me(I) and 7,8-di MeO(I); group *B* includes 7-OH(I) and 8-OH(I); group *C* includes 6,7-di OH-4-Me(I) and 7,8-di OH-4-Me(I).

In a run, 50 ml. $5{\cdot}636 \times 10^{-4}$ M solution of one of the above coumarins in 10 per cent methanol (by volume) was passed through the column at 5 ml./min and the sorbed solute on the column was eluted. The effluent was collected in samples and estimated for the solute content by ultra-violet absorption[4]. Group *A* compounds were eluted by 10 per cent methanol. Group *B* compounds were not eluted by 10 per cent methanol, but were slowly eluted by N/10 HCl in 10 per cent methanol. Group *C* compounds were not eluted by 10 per cent methanol or by N/10 HCl in 10 per cent methanol, but were eluted by N/100 HCl in methanol. It is therefore possible to separate binary and ternary mixtures containing not more than one compound from each group.

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BIOCHEMISTRY

Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age

A number of recent reports[1,2] have indicated that the *in vitro* storage of human red blood cells leads to an increased electrophoretic mobility of some enzymes (for example, glucose-6-phosphate dehydrogenase, acid phosphatases) when compared with the mobility of the same enzyme obtained from fresh blood. This finding parallels that previously reported for haemoglobin derived from fresh or stored red cells[3].

The availability of several methods for the separation of young and old red blood cells or of their lysates from a red blood cell population[4–6] prompted us to investigate the electrophoretic behaviour of some enzymes derived from erythrocytes of different ages. This communication presents results of preliminary experiments indicating an altered electrophoretic mobility of some erythrocytic enzymes as a function of the age of the red cell from which they are derived.

It was also found that this altered electrophoretic behaviour is not shared by all erythrocytic enzymes, nor even necessarily by enzymes having the same substrate specificities but originating in different aged erythrocytes of other species. The implications of these experiments are briefly discussed.

100 ml. of human blood, collected in EDTA or acid–citrate–dextrose solutions, were washed six times with isotonic saline, great care being taken to remove the buffy white blood cell layer. The washed red cells were then subjected to the serial osmotic haemolysis procedure of Simon and Topper[6] in which lysates from young and old erythrocytes are obtained separately. The smallest possible lysing volumes were used so as to have a high enzyme content per unit volume. Lysates were centrifuged at high speed (13,000*g* for 10 min) to remove the stroma. The haemolysates corresponding to the reticulocytes were not examined.

Control experiments on the behaviour of white blood cells in the serial osmotic haemolysis procedure indicated that the contribution of enzymes by residual, contaminating white blood cells in the red blood cells fractionated was small and most probably did not affect the results described here. Further experiments, however, using other separation procedures for young and old erythrocytes[5] are in progress to confirm our results by another, independent method.

Glucose-6-phosphate dehydrogenase (G-6-PD) and glutamic-oxaloacetic transaminase (GOT) activities were assayed by standard spectrophotometric methods[7–9] on the lysates of the youngest and older human erythrocytes. One unit of enzyme is defined as that amount required for a $\Delta O.D._{340\ m\mu}$ of 0·001/min/ml. of lysate. For comparative purposes samples of blood were stored at 4°–5° C for up to four weeks. The latter samples were washed with saline after storage, lysed and enzyme activities determined as indicated above.

Starch-gel electrophoresis of G-6-PD was carried out according to the method of Fildes and Parr[1] except that, at these authors' suggestion, a 0·01 M phosphate buffer at *p*H 7·5 was substituted for the *tris* buffer described in their paper. GOT was electrophoresed following the method of Schwartz *et al.*[10], but using a 0·01 M phosphate buffer at *p*H 7·3.

In all the electrophoretic runs the number of enzyme units of each lysate applied was identical to that of the fraction to which it was compared. About 10 units (as defined here) were applied for both G-6-PD and GOT.

Fildes and Parr[1] reported that G-6-PD has an increased electrophoretic mobility when the red blood cells from which it is derived have been stored. We have repeated these experiments and confirm their findings. The

difference in electrophoretic mobilities between fresh and stored G-6-PD is of the order of 10 per cent, although in some cases the difference has been appreciably greater. Our examination of GOT, obtained from fresh and stored red cells, revealed a smaller alteration in the electrophoretic mobility of this enzyme. These results, together with the described increase in electrophoretic mobilities of erythrocyte acid phosphatases[2], lead to the question of whether all erythrocytic enzymes manifest this behaviour. Examination of the electrophoretic behaviour of lactic acid dehydrogenase (LDH) isozymes[11] reveals that there is no alteration in the electrophoretic mobilities of these isozymes as a function of red blood cell storage *in vitro*. Thus, apparently, only certain enzymes display this behaviour.

Visualization after electrophoresis of G-6-PD and GOT derived from young or old erythrocytes reveals that the alteration in mobilities of these enzymes is not solely a function of their storage *in vitro*. These enzymes show a similar alteration in electrophoretic mobility on ageing *in vivo* (Figs. 1*A* and *B*). This may be analogous to results reported for haemoglobin in which haemoglobin A_3 (the old haemoglobin) displays an increased electrophoretic mobility[12] when compared with its younger counterpart (Hb A).

A number of suggestions have been made as to the possible cause of this increased mobility of haemoglobin on *in vivo* or *in vitro* ageing, one of which is that a mixed disulphide with glutathione is formed[13] as a consequence of the diminished enzyme activities (for example, glutathione reductase) in older red cells. A number of other possibilities have also been discussed[14] in view of the fact that a mixed disulphide formed between haemoglobin and glutathione *in vitro* has some properties that differ from those of Hb A_3 (ref. 3). In any event, whichever of the suggested reactions accounts for the alteration of the electrophoretic mobility of haemoglobin as a function of the age of the cell from which it is derived may well be similarly responsible for the altered mobilities of the G-6-PD and GOT, since all these proteins are contained in the same changing cellular environment.

That the reaction leading to the increased electrophoretic mobilities of erythrocytic enzymes is not one that occurs with all these enzymes is shown by the fact that LDH isozymes display neither a storage effect nor do they have altered mobilities as a function of the age of the red cell from which they are obtained[11].

Finally, investigation of the behaviour of G-6-PD from horse erythrocytes indicates that this enzyme (which in the horse consists of two isozymes) shows neither a storage effect nor an altered electrophoretic mobility as a function of the age of the red blood cell from which it is derived.

It may well be that those enzymes which have certain reactive groups on their surface (for example, sulphydryl) react with some reagent (for example, glutathione) as the cell ages and display the altered mobility observed on electrophoresis. Conversely, those enzymes not possessing such groups in readily accessible positions do not. If this is so, it might also serve as an indicator of this type of different molecular structure of some enzymes having similar substrate specificities but derived from different species (for example, G-6-PD from human and from horse). We are at present investigating some of these possibilities.

In conclusion: the examination of the electrophoretic mobilities of G-6-PD, GOT and LDH from young or old human erythrocytes reveals that G-6-PD and GOT derived from the older cells have increased electrophoretic mobilities while the LDH isozymes do not.

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Note added in proof. Experiments conducted since this communication was submitted for publication in which human and horse haemolysates were reacted with oxidized glutathione and the resulting electrophoretic mobilities of the aforementioned enzymes compared to those from unreacted lysates indicate that human G-6-PD and GOT have increased electrophoretic mobilities after reacting with glutathione while human LDH and horse G-6-PD have not.

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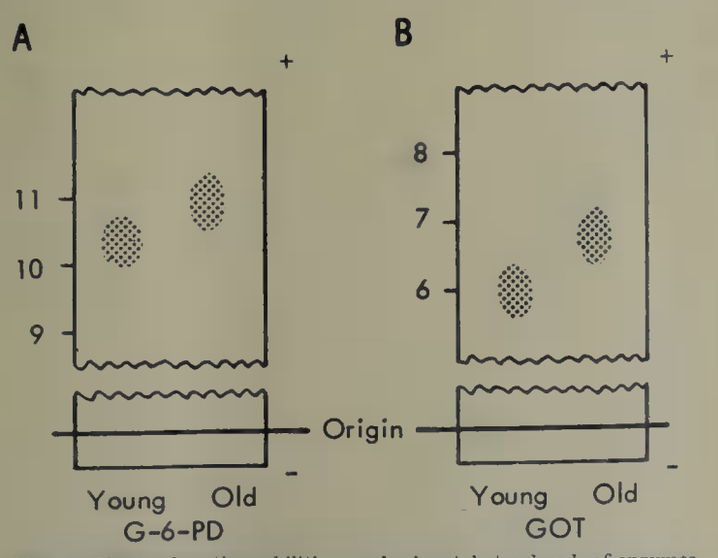

Fig. 1. Electrophoretic mobilities, on horizontal starch-gel, of enzymes derived from young or old human erythrocytes. *A*, Glucose-6-phosphate dehydrogenase, 0·01 M phosphate buffer, 5 V/cm, 16 h. *B*, Glutamic-oxaloacetic transaminase, 0·01 M phosphate buffer, 6 V/cm, 16 h. Distance of migration in cm

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Deterioration of Stored Inulin Solutions

While using a batch of inulin for determination of glomerular filtration rate in man, we found that some ampoules were giving much lower serum inulin-levels than were expected from the dose given. The inulin had been stored in the dark at 20°–25° C, in sealed ampoules, and from the batch number it was established that it had been manufactured thirteen years ago by Thomas Kerfoot and Co., Barnsley. We investigated the deterioration of inulin with time by determining the inulin content by the acid hydrolysis and resorcinol technique[1] in fifteen ampoules of the thirteen-year-old batch of inulin, in seven ampoules of inulin one year old and in inulin recrystallized from the one-year-old batch, after washing with distilled water at 20° C. The ages of the inulin batches were kindly provided by the manufacturers.

Table 1 shows the results of total inulin content; taking recrystallized inulin as 100 per cent, the thirteen-year-old

'Inulin' Content of Stored Inulin Ampoules

Inulin sample	Per cent inulin determined by acid hydrolysis–resorcinol technique[1]	
Recrystallized inulin	100	
13-year-old inulin:		
Ampoule 1	54·8	
2	75·8	
3	60·6	
4	69·5	
5	74·1	
6	64·0	
7	83·0	
8	64·0	
9	78·2	
10	92·0	
11	81·0	
12	75·6	
13	74·5	
14	75·3	
15	72·6	
Mean	73·0	*S.D.* 9·07
1-year-old inulin:		
Ampoule 1	83·9	
2	87·1	
3	89·2	
4	89·7	
5	90·3	
6	92·8	
7	89·2	
Mean	88·9	*S.D.* 2·36

Table 2. Amount of Reducing Substances in 10 per cent Inulin Solutions expressed as Fructose (ref. 2)

Type of inulin	Fructose g/100 ml. in a 10 per cent solution of inulin
(1) 13-year-old batch. First ampoule	5·05
(2) 13-year-old batch. Second ampoule	4·83
(3) 1-year-old batch. First ampoule	0·97
(4) 1-year-old batch. Second ampoule	1·42
(5) Recrystallized inulin	0·32

batch had a mean inulin content of 73·0 per cent *S.D.* 9·07, and the one-year-old batch had a mean inulin content of 88·9 per cent *S.D.* 2·36. Because the acid hydrolysis–resorcinol technique measures fructose and non-inulin fructose polymers, there must be considerable degradation of inulin to products other than fructose during prolonged storage. The true picture of how much inulin remains unaltered can be seen indirectly from Table 2, in which reducing substances were estimated by Benedict's quantitative method[2] in samples of thirteen-year-old inulin, one-year-old inulin and recrystallized inulin. It will be seen that about 5 g per cent of reducing substances (expressed as fructose) was present in 10 per cent inulin solutions thirteen years old, whereas 10 per cent inulin solutions one year old have between 1 and 1·5 g per cent reducing substances (as fructose) and 10 per cent solutions of recrystallized material contain 0·3 g per cent of reducing substances.

This work suggests that even one-year-old inulin contains appreciable amounts of non-inulin reducing substances, presumably fructose or fructose polymers; these may give falsely high values for plasma inulin re-absorption by the tubules[3]; a considerable error may be introduced into the measurement of glomerular filtration rate. It is concluded that for inulin-clearance measurements, solutions of inulin freshly prepared from recrystallized inulin should be used, and that inulin samples more than one year old should be discarded.

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Interaction between Chlorophyll and Hydroquinone

Chlorophyll has a unique characteristic in being photo-oxidized or photo-reduced by reactants which can serve as electron acceptors or donors to light-activated chlorophylls[1]. The photo-reduction of chlorophyll occurs when ascorbic acid is the reductant[2]. Quinones act as electron acceptors for the photo-oxidation of chlorophyll[3], but hydroquinones have not been shown to function as electron donors to the excited chlorophyll. It is known that hydroquinones are photo-oxidized by oxygen in chlorophyll *a* and *b* solutions[4]. The work recorded here consists of an experimental investigation of the direct interaction between excited chlorophyll and hydroquinone. The effect of oxygen on this system is also shown in this communication.

Chlorophyll *a* was prepared and purified by Zscheile's method[5]. Hydroquinone (Merck) was recrystallized four times from methanol solution. Photovoltaic changes of chlorophyll *a* with and without hydroquinone in methanol were measured by means of a Kintel digital voltmeter, model 456 *B*, with read-out, model 473 *A*. The photovoltaic cell consisted of a platinum electrode with a saturated calomel electrode as reference. The cell was irradiated through a collimating lens at a distance of 25 cm by a 1 kW-tungsten projection lamp. A Corning red filter *CS*-2-61 was used together with a 2 per cent copper sulphate solution. The solutions were flushed before and during measurements with nitrogen gas pretreated with a chromous chloride solution. Electron spin resonance absorptions were determined by a Varian *V*-4502 equipped with 100-kc/s field modulation. The cavity of the EPR spectrometer was irradiated in the same way as in the measurement of photovoltaic changes. The solutions prior to irradiation were degassed on a vacuum line as described in the studies of the triplet state of chlorophyll[6].

Fig. 1 shows the small photovoltaic change with chlorophyll *a* ($7{\cdot}4 \times 10^{-6}$ M) in de-aerated methanol. Addition of hydroquinone to the same solution at a concentration of 10^{-4} M greatly enhances the photovoltaic change. The redox-potential of this system decreases in light and reconverts to approximately the initial value when the light is turned off. This reversible change indicates an electron

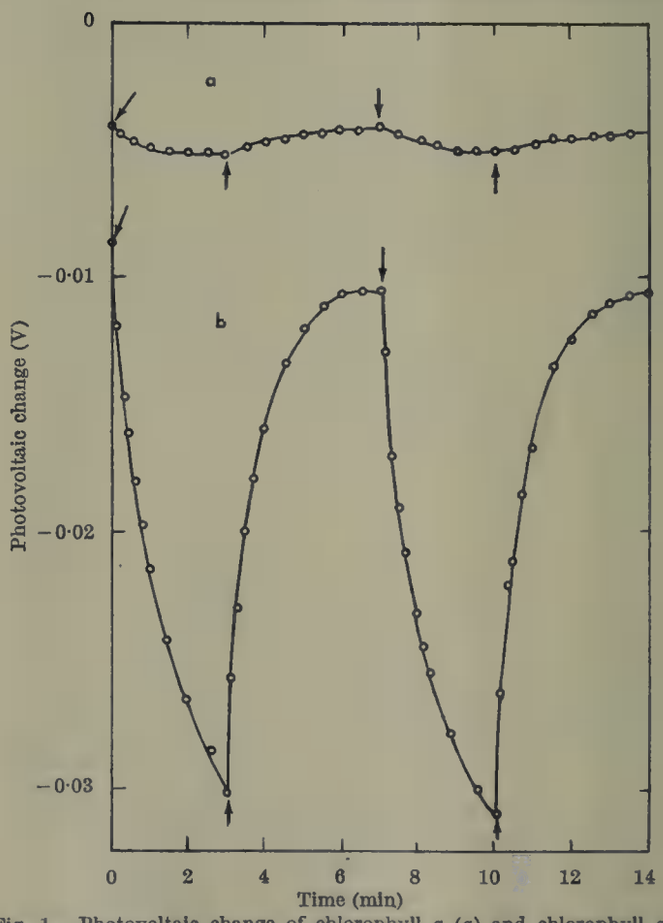

Fig. 1. Photovoltaic change of chlorophyll *a* (*a*) and chlorophyll *a*–hydroquinone (*b*) in methanol: $7{\cdot}4 \times 10^{-6}$ M chlorophyll *a* and 10^{-4} M hydroquinone. Downward-pointing arrows show light on and upward-pointing arrows light off. Photovoltaic change is represented by $E_h - E_{cal}$, where E_h and E_{cal} are the redox-potential of the system and the electrode-potential of saturated calomel electrode, respectively

exchange between excited chlorophyll and hydroquinone. The negative change of the redox-potential on irradiation suggests that chlorophyll undergoes photo-reduction and its couple is responsible for the photovoltaic effect. The electron spin resonance measurements exhibited a greater rate of electron transfer than that found in the photovoltaic study. This difference is attributed to the slow electrochemical process at the electrode responding to the redox-reaction involving excited molecules in solution.

Electron spin resonance investigations showed that this photo-reversible change is due to one-electron transfer from hydroquinone to chlorophyll. Degassed solutions of chlorophyll *a* ($4{\cdot}5$–$4{\cdot}9 \times 10^{-4}$ M) with hydroquinone (10^{-2} M) in methanol exhibit two types of electron spin resonance signal when irradiated with red light as shown in Fig. 2*a*. One shows a hyperfine structure, which is characteristic of *p*-benzosemiquinone free radical, and the other is a broader signal probably from reduced chlorophyll. Fig. 3*a* illustrates a characteristic rapid rise and a quick decay of the signals.

In the presence of air, two similar types of signal were obtained and both were greatly enhanced (Fig. 2*b*). One, a single absorption, is a little broader than that in the air-free system. The other is the same as the aforementioned signal having the hyperfine structure. The time to attain saturation of the signals was slower in the aerated solution than that found for the de-aerated solution as shown in Fig. 3*c*, especially the signal with the hyperfine structure which grew slowly during irradiation even after the broad signal had reached its saturation. The hyperfine signal decayed rapidly, while the decay of the broader signal was slower. With aerated chlorophyll *a*–methanol solution without hydroquinone, a smaller broad electron spin resonance signal is observed which is similar to the broad signal of the aerated chlorophyll *a*–hydroquinone solution. This indicates that these broad signals originate from an interaction between excited chlorophyll and oxygen. Chlorophyll *a* in methanol solution in air undergoes a permanent oxidation as evidenced by the decrease in electron spin resonance signal with continuous irradiation. The electron spin resonance decay of this solution was intermediate between that of chlorophyll *a* with hydroquinone and that with hydroquinone and oxygen (Fig. 3*b*). Calvin also showed this kind of electron spin resonance absorption in aerated methanol extract from *Chlorella*[7].

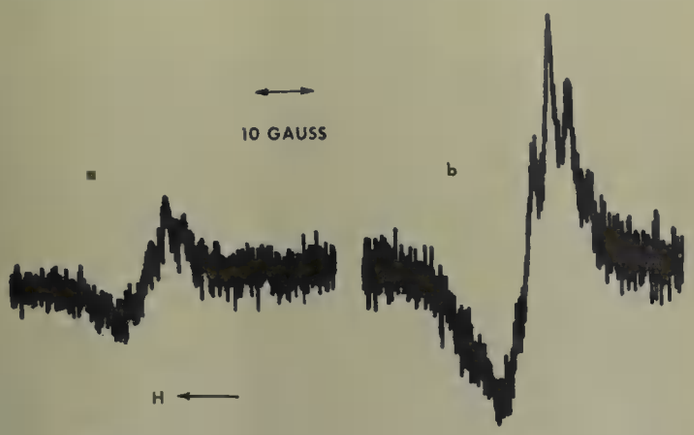

Fig. 2. Light-induced electron spin resonance spectra of chlorophyll *a*–hydroquinone system in methanol in the absence of oxygen, taken immediately after irradiation (modulation amplitude: 2·4 gauss) (*a*) and in the presence of oxygen, taken after irradiation for 7 min (modulation amplitude: 0·6 gauss) (*b*): $4{\cdot}6 \times 10^{-4}$ M chlorophyll and 10^{-2} M hydroquinone

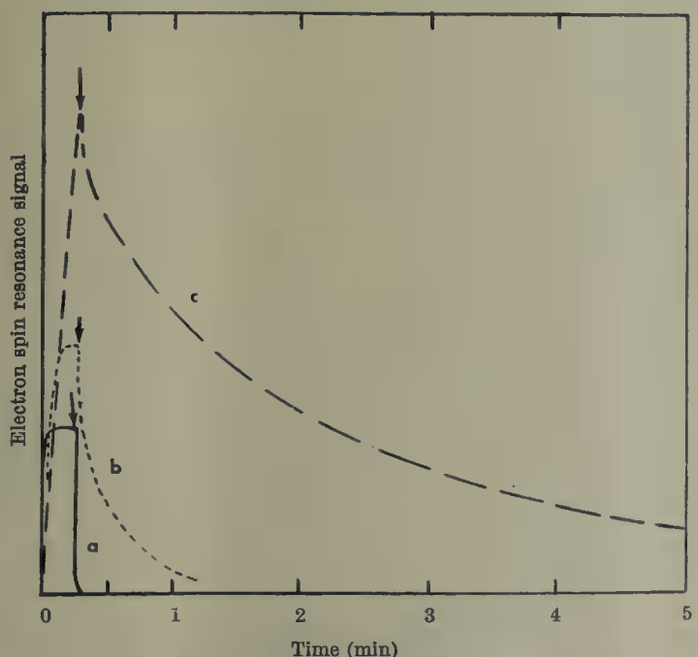

Fig. 3. Rise and decay curves of electron spin resonance signals of chlorophyll *a* ($4{\cdot}9 \times 10^{-4}$ M)–hydroquinone in the absence of oxygen (*a*), chlorophyll *a* ($4{\cdot}5 \times 10^{-4}$ M) in air (*b*), and chlorophyll *a* ($4{\cdot}5 \times 10^{-4}$ M)–hydroquinone in air (*c*), in methanol: 10^{-2} M hydroquinone (modulation amplitude: 4·8 gauss). Downward-pointing arrows show light off after irradiation for 15 sec

Small photovoltaic changes obtained in anaerobic solutions of chlorophyll *a* in methanol are attributed to a reversible photo-bleaching of chlorophyll. This is consistent with a mechanism proposed by Livingston and Stockman[8], where a very small amount of chlorophyll is reversibly photo-reduced by complexed methanol molecules.

The interaction between excited chlorophyll and hydroquinone shown in anaerobic solutions is significant for specific aspects of chlorophyll photochemistry. Very recently chlorophyll-photosensitized reduction of cytochrome *c* has been shown by chloroplasts or isolated chlorophyll *a* in the presence of hydroquinones. These reactions have been assumed to proceed via an initial photochemical reduction of quinone present as an impurity in hydroquinone[9]. Flash-illumination of chlorophyll solution containing reduced ubiquinone induced a rapid absorption change at 430 mμ, followed by a rapid decay which is slower than a decay of triplet state[10]. The work recorded here shows that the direct reaction of excited chlorophyll with hydroquinone is possible. Our investigation on the role of hydroquinone in the photoreactions occurring in chloroplasts is now in progress.

The excited chlorophyll molecule undergoes reactions with oxygen to form an unstable, reactive complex[11,12]. This occurs with the formation of free radicals and the subsequent permanent oxidation of chlorophyll[13]. In the presence of hydroquinone, the free radicals that are formed in the quick reaction of excited chlorophyll with oxygen probably undergo further reactions with hydroquinone to produce *p*-benzosemiquinone-free radicals in the oxidation of hydroquinone.

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Biosynthesis of Triiodothyronine Sulphate by Beef Thyroid *in vitro*

It is well recognized that the thyroid gland contains the L-amino acids, triiodothyronine (T_3), thyroxine (T_4), monoiodotyrosine (MIT), diiodotyrosine (DIT) and iodinated thyronines which are free (unconjugated) but are bound to thyroglobulin. The release of these iodinated amino-acids occurs after digestion by protein-hydrolysing enzymes or chemicals, but the possibility of observing any T_3 and/or T_4 sulphate conjugates *per se* in thyroid tissues seems unlikely since hydrolytic procedures would have precluded their isolation.

The conversion of triiodothyronine (T_3) to triiodothyronine sulphate (T_3S) presumably occurs in the liver[1] since Roche, Michel, Closon, and Michel demonstrated the presence of T_3S in the bile and plasma of thyroidectomized rats. However, the well-established evidence for extrahepatic mammalian steroid sulphokinase activity in endocrine glands such as adrenal[2,3] and ovary[4] stimulated an investigation of T_3 sulphokinase activity in mammalian thyroid tissues.

Fresh beef thyroid glands were homogenized at 4° C in 0·25 M sucrose, containing 0·05 M *tris* (*tris* hydroxymethyl aminomethane) and 0·002 M EDTA (ethylenediaminetetraacetate) at *p*H 7·5. The crude homogenate was filtered through cheese cloth and fractionated by ultracentrifugation according to the method of Fischer, Schultz and Oliner[5]. The fractions I–IV (see Table 1) were stored frozen until assayed.

The assay mixture included 100 μmol imidazole HCl buffer, *p*H 7·0, 12·5 μmol $MgCl_2$, 1·0 μmol triiodothyronine (Sigma), 10 μmol cysteine (neutralized), 10 μmol K_4ATP, 10 μmol K_2SO_4, 0·2 ml. yeast sulphate-activating enzyme[6] and 0·2 ml. thyroid sulphokinase fraction in a final volume of 1·0 ml. Incubation was carried out for 35 min in air at 37° C in the Dubnoff metabolic shaker.

Control flasks included zero time, and mixtures incubated without various components including enzymes. The T_3S was determined by the methylene blue method of Nose and Lipmann[7]. A standard curve was prepared by the use of the ammonium T_3S which was synthesized by the method of Fieser[8]. Protein was measured spectrophotometrically by the method of Gornall and colleagues[9].

Table 1. T_3 Sulphokinase Activity of Beef Thyroid

Fractional ultracentrifugation		mμmol T_3S formed per mg of protein
Fraction I	Filtered crude homogenate	92
Fraction II	700*g* for 10 min supernatant	161
Fraction III	12,000*g* for 10 min supernatant	182
Fraction IV	105,000*g* for 60 min supernatant	216

The results of a typical incubation are listed in Table 1. The identification of ammonium T_3S sulphate in the incubation mixture followed the addition of concentrated ammonium hydroxide and extraction with *n*-butyl alcohol. Identical mobilities of the unknown and standard were observed after descending paper chromatography in the *n*-butyl alcohol, 2 N, NH_4OH system and paper electrophoresis in the $(NH_4)_2CO_3$, 0·05 M, *p*H 9 according to the methods of Roche *et al.*[1]. The T_3S was located by positive ninhydrin stain and Schneider, Lewbart modification of the rhodizonic acid test for sulphate ions[10].

The data indicate that T_3 sulphokinase activity is present in beef thyroid tissue and that the highest enzymatic activity is in the microsome-free supernatant. It is of interest that sulphokinase activity is also highest in the hepatic and adrenal microsome-free fraction, that is 105,000*g* supernatant. The thyroid T_3 sulphokinase activity remained constant for at least 3 months despite frequent freezing and thawing.

Preliminary studies suggest that beef thyroid microsome-free supernatant synthesizes T_4 sulphate (T_4S) in the same order of magnitude as T_3S (210 mμmol per mg of protein per 35 min). Additional studies are in progress to purify T_3 and T_4 sulphokinase(s) and to study the enzyme kinetics involved.

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PHYSIOLOGY

Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat

It has been shown by Muschaweck and Hajdú[1] that in the dog, at doses of 10 mg/kg body-wt. and more, 'Furosemid' (4-chloro-*N*(2-furylmethyl)-5-sulphamoyl-anthranylic acid) markedly increases the excretion of water, sodium and chloride. These authors also showed that the increase in chloride excretion was proportionally greater than that of sodium and water. On the other hand, Karger and Nagel[2] found a reduced permeability to anions, especially chloride, in the frog skin after addition of 'Furosemid' at concentrations of 25–500 mg/l. to the bathing solution, as evidenced by increased transmembrane potential differences without change in the short circuit current. Deetjen[3] obtained proximal and distal tubular fluid samples in rats with an intravenous infusion of 2 mg/kg/min and observed significantly decreased proximal fluid/plasma (F/P) inulin ratios, as well as ratios below 2 in the distal tubule, concluding that 'Furosemid' depresses water and salt re-absorption in the proximal tubule and ascending limb of Henle's loop. Clearance studies by Suki, Rector and Seldin[4] also indicated that 'Furosemid' might have an effect on the activity of the re-absorptive mechanisms of the proximal tubule and the ascending limb of Henle's loop.

Having this evidence in mind, we decided to investigate the fate of chloride along the nephron of the rat kidney, using micropuncture methods as described by Windhager and Giebisch[5]. Extreme care was taken in the collection of distal tubular samples, using rather long oil blocks (at least four times the tubular diameter), and checking the direction of flow by subsequent microdissection. Chloride and inulin were measured simultaneously, the first by the second coulometric method of Ramsay *et al.*[6], the second by a modification of the microanthrone method of Hilger *et al.*[7]. 'Furosemid' was infused in saline (0·05 ml./min) at a rate of 0·8 to 1 mg/kg/min, sufficient to produce a marked diuresis. In parallel experiments the same measurements were done in control rats receiving saline infusion (0·05 ml./min) and in animals receiving a 4 per cent sodium chloride infusion at a rate of 0·1 ml./min.

Mean glomerular filtration rate (GFR) was 3·21 ml./kg/min in the 'Furosemid' group, 4·22 in the control group, and 5·77 in the 4 per cent sodium chloride group. Mean urinary *p*H was 6·32 (range 5·95–6·71) in the control group, 6·50 (5·99–6·83) in the 4 per cent sodium chloride infused, and 5·26 (4·76–5·82) in the 'Furosemid' group.

In Table 1*a* mean chloride F/P ratios in proximal and distal tubules, as well as chloride U/P ratios, are shown

Table 1

(*a*) Chloride F/P ratios*

	Proximal			Distal			Urine		
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Control	1·29	1·07–1·43	(12)	0·30	0·12–0·56	(15)	0·29	0·05–1·16	(11)
NaCl 4%	1·24	1·15–1·41	(8)	0·46	0·25–0·78	(10)	2·34	2·01–2·80	(7)
'Furosemid'	1·29	1·20–1·41	(10)	1·27	1·13–1·47	(15)	1·45	1·36–1·65	(15)

(*b*) Inulin F/P ratios

	Proximal			First 1/2 distal			Urine		
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Control	2·08	1·15–3·78	(11)	6·01	3·73–7·80	(10)	201·2	56–356	(12)
NaCl 4%	1·39	1·04–1·85	(8)	2·28	1·87–3·28	(9)	17·5	8·8–32·4	(7)
'Furosemid'	1·75	1·21–2·51	(7)	4·51	2·97–5·92	(9)	7·6	5·2–10·7	(15)

* Not corrected for an experimental ultrafiltrate/plasma ratio of 1·06.

in the 'Furosemid' and in the control groups. It can be seen that all three groups show similar proximal ratios, above unity. In the distal tubule, however, there is a marked difference between the control groups, having ratios significantly below unity, and the 'Furosemid' group, showing ratios consistently above one, similar to those observed in proximal tubules. There is a considerable rise in chloride ratios between distal tubules and collecting duct urine in the group receiving 4 per cent sodium chloride, but only very little further increase in the 'Furosemid' group. In Table 1*b* the corresponding inulin concentration ratios are seen. The proximal tubular inulin ratios are somewhat reduced, though not so much as those in the experiments of Deetjen[3], probably due to our lower rate of infusion of 'Furosemid'. A larger reduction is observed in the early distal samples. On the other hand, it is noteworthy that there is little difference between the distal and urine inulin ratios in the 'Furosemid' group, as compared with the 4 per cent sodium chloride infused rats. The inulin ratios obtained in the latter group are similar to those obtained by Giebisch *et al.*[8]. Electrical potential difference measurements with glass microelectrodes in the 'Furosemid' infused rats showed proximal and distal transtubular potential differences within the range of controls (means −19·0 and −50·6 mV, respectively).

The results obtained in these experiments show that the mechanism of diuresis in rats undergoing 4 per cent sodium chloride infusion and during 'Furosemid' infusion is a quite different one, in spite of roughly similar overall chloride excretion rates (mean urinary Cl/inulin excretion rates of 0·13 in sodium chloride infused, and 0·19 in 'Furosemid' infused rats).

It appears that at our infusion rates of 'Furosemid' there is a relatively small inhibition of proximal and distal tubular fluid re-absorption. The major effect observed appears to be an impairment of the re-absorption of sodium chloride in excess of water in the ascending limb of Henle's loop, a process which constitutes the 'single effect' for the counter-current multiplier system of the renal medulla[9]. As shown by the high early distal chloride ratios in the 'Furosemid' group, the normally occurring hypotonicity of the tubular fluid at this site is absent, a further indication of impairment of the transport mechanisms in the ascending limb of Henle's loop. From our results, it appears that, due to inhibition of chloride and fluid re-absorption at this site, the build-up of a medullary salt concentration gradient might be impaired, as evidenced by the reduced further increase in the chloride and inulin F/P ratios between distal tubule and final urine. These experiments suggest, therefore, that 'Furosemid' in doses of 0·8–1 mg/kg body-wt./min acts preferentially on the sodium chloride transport system of the ascending limb of Henle's loop and the early distal convolution, preventing the establishment of a considerable concentration gradient for sodium chloride along these tubular segments.

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Feeding and Reproduction of Rats fed Calcium Cyclamate

With the increased production and consumption of calcium cyclamate as a sweetening agent in foods for dietary uses, a series of feeding trials on rats was initiated at the request of, and under contract with, the Sugar Research Foundation, Inc.

Earlier work in this area was reported in 1951. Fitzhugh *et al.*[1] using rats as test subjects fed sodium cyclamate at levels up to 5 per cent in the diet for a two-year period. At levels below 1 per cent sodium cyclamate displayed little if any effect in the rat. At a level of 5 per cent in the diet, weight loss accompanied by moderate diarrhoea was reported. Histopathological examination of the animals was normal.

As this early work was performed with sodium cyclamate, and as the human consumption of calcium cyclamate has greatly increased during the intervening years, additional feeding trials appeared in order. The use of calcium cyclamate in human dietaries where reduced calorie intake is important led to the inclusion of feeding trials where calorie intake was restricted.

Weanling rats were placed on dietaries containing 5 and 10 per cent calcium cyclamate. Growth, food and water intake, and biochemical parameters were observed. After eight weeks on test, half the animals from each group were placed on restricted intake of food. These latter animals were limited to 60 per cent of the calorie intake of the animals receiving *ad lib.* feeding.

At eight weeks the females from each group were mated with males from their respective groups. Females were allowed to deliver their young and raise them to weanling stage (21 days). Following a rest period the females were mated a second time and allowed to raise their young to weanling age.

Haemoglobin and urine examinations were conducted at 6 and 8 months with selected animals. Selected

animals from the control group and the group receiving 10 per cent calcium cyclamate were killed at 6 months and examined histophathologically.

So far (9 months), body-weight and food consumption data have illustrated an effect of feeding calcium cyclamate in the diet, whether on full feed or on restricted feed intake. Animals receiving 10 per cent calcium cyclamate consumed approximately 10 per cent more diet than control animals. This increase in food intake compensated for the non-caloric cyclamate in the diet. Thus the 10 per cent calcium cyclamate rats' calorie intake was equal to the control animals. Despite equal calorie intake animals receiving calcium cyclamate at the 10 per cent level grew at a rate 20–30 per cent less than control animals. This growth depression results from the inclusion of the calcium cyclamate in the ration, but whether this is due to toxicity *per se* or an interference with nutrient absorption in the gastro-intestinal tract is being determined. Possible effect on metabolic rate is being examined. The same growth depression was noted to a lesser degree in animals receiving 5 per cent calcium cyclamate in their diet.

Animals in all groups were in good health. The faeces of animals receiving calcium cyclamate in the diet were soft and moist. Initial diarrhoea during the early weeks of the experiment disappeared after 8 weeks on test. No remarkable variation was noted among the rats during haematological or urine examination. Animals receiving calcium cyclamate consumed 25–40 per cent more water daily.

Animals receiving *ad lib.* feed conceived and were able to raise their young to weaning (21 days) during both breeding trials. In the first litters the control young averaged 50 g in weight at 21 days of age, the 5 per cent calcium cyclamate young averaged 42 g, and the 10 per cent calcium cyclamate young averaged 32 g.

In the second litter, control young averaged 52 g, 5 per cent calcium cyclamate young 45 g, and 10 per cent calcium cyclamate young 32 g.

Reproduction investigations using animals receiving restricted or limited food intake were carried out. These animals conceived, bore their young, but were unable to maintain them beyond 5 days of age.

In an effort to understand the reduced growth rates in animals receiving calcium cyclamate in the diet despite equal calorie intake a balance study was set up. This investigation indicated complete utilization of the ration based on nitrogen retention and faecal and urinary nutrient excretion.

Other parameters to understand these effects, including possible increased metabolic rates and ultimate fate of cyclamate, are being investigated.

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Carbon Dioxide Production of Whole Blood *in vitro*

A recent publication[1] has shown the mean oxygen consumption of whole blood *in vitro* at 37° C to be $8{\cdot}5 \times 10^{-5}$ ml./ml./min, and the mean rate of increase of P_{CO_2} to be 0·11 mm mercury/min. The addition of sodium fluoride was found to reduce the oxygen consumption by 26 per cent and to prevent completely the rise of P_{CO_2}. The authors commented that their findings were paradoxical "because oxygen could not be consumed without production of carbon dioxide".

We believe this statement to be incorrect. Working in separate centres, we have reached the conclusion that carbon dioxide production of whole blood *in vitro* at normal P_{O_2} is negligible compared with its oxygen consumption. We have, in fact, found the carbon dioxide content of blood to fall during storage, although our values for oxygen consumption are of the same order as those of Lenfant and Aucutt[1].

Table 1

	Temp. (° C)	Method	Rate of fall of O_2 content ml./ml./min	Temp. (° C)	Method	Rate of fall of CO_2 content ml./ml./min
Capel and Fletcher	37	Modified Haldane	$10{\cdot}4 \times 10^{-5}$	30	Modified Haldane	$2{\cdot}1 \times 10^{-5}$
Nunn	38	Polarography at 100% saturation of haemoglobin	$10{\cdot}5 \times 10^{-5}$	38	Van Slyke	$5{\cdot}6 \times 10^{-5}$

Our results suggest a negative respiratory quotient for whole blood at normal P_{O_2} although we agree closely with the rate of rise of P_{CO_2} reported by Lenfant and Aucutt[1]. We believe that this rise cannot be due to carbon dioxide production and suggest that it is due to the production of non-gaseous acids. We have found that blood stored at 37° C develops a metabolic acidosis of the order of 0·03 m.equiv./l./min, a figure which is in close agreement with the rate of increase of lactic acid[2]. It can easily be shown by the addition of lactic acid to blood (*in vitro*) that the production of lactic acid is sufficient to account for the rise in P_{CO_2} without invoking the production of carbon dioxide.

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Transplantation Experiments on Placental Ageing

Krohn[1] has recently re-emphasized the role that transplantation studies can play in the elucidation of the process of ageing in organs and tissues. He has shown that skin and ovary have intrinsic life cycles, to a large extent independent of central mechanisms. These techniques have not been successfully used in the study of rapidly ageing transient tissues like the placenta. Earlier transplantation studies of placenta have always used allogeneic tissues, since the importance of genetic differences in such experiments was not appreciated[2]. The present experiments, to be described in detail elsewhere, were designed to demonstrate the evolution of syngeneic transplants of mouse trophoblast with age.

Adult mice of the highly inbred strains *C3H* and *C57* were mated (*C3H* female × *C3H* male, *C3H* female × *C57* male, *C57* female × *C3H* male). The gestational age of each pregnancy was calculated from the day of the appearance of a copulation plug. The pregnancies were interrupted on gestational days 2, 7, 10, 15, 18 and 20. Recipient animals were adult male mice syngeneic to the donor tissue in all cases. At two and seven days, the fertilized ova and the ectoplacental cone were transplanted into the spleen or under the kidney capsule as previously described[3]. The placental rims of the older pregnancies were divided into 1-mm fragments and implanted into the spleen and under the kidney capsule. The recipients were killed 5, 10, 15 and 20 days after transplantation, and the tissue fixed in formalin. Haematoxylin and eosin staining was routinely carried out.

Trophoblast underwent active proliferation from the implanted ova and ectoplacental cones of the 2- and 7-day-old pregnancies and it was actively invasive of host tissue. A large amount of haemorrhage was present from eroded host vessels by five days after transplantation. Such transplanted trophoblast cells were of large size with long cytoplasmic processes, giant nuclei and prominent nucleoli. Living cells from such transplants could occasionally be found as long as 25 days after transplanta-

tion, but most frequently cells survived for only 15–20 days. Host cellular reaction was minimal. No definite cytolysis or phagocytosis of host tissue as reported by Kirby[4] was seen nor was there any ingrowth of host capillaries into the graft. Trophoblast-free haemorrhagic cysts interspersed with shreds of eosinophilic hyalin material remained after cell death.

In contrast, no proliferation or invasion of host tissue was present in any of the transplants performed after 10 days gestation. Haemorrhage was elicited by the 10-day gestational placental transplant, but its appearance was delayed until 10 days after transplantation. No haemorrhage was present in any transplant older than 15 days gestation. Vascularization of the transplant did not take place.

A cell pattern similar to that of the original transplanted placental fragments could be seen in the older implants removed 5 days after transplantation. Such patterns disappeared from specimens taken 10 days after implantation. Generally the smaller trophoblastic cells peculiar to the labyrinth of the placenta disappeared with time and the proportion of giant cells increased. In the specimens taken 10–15 days after transplantation, the vacuolized eosinophilic cytoplasm of the remaining cells tended to coalesce with the loss of cell boundaries. Soon afterwards, the giant cells became swollen or disintegrated. The cytoplasm then lost its vacuoles and became hyalinized. In the 15–20-day specimens, viable cells were rare or absent and eosinophilic hyaline bands frequently occupied the entire implant. Extensive hyalinization was present as early as 5 and 10 days after transplantation in the 18-day and 20-day gestational transplants.

In conclusion, it would appear that the transplanted mouse trophoblast has an intrinsic life cycle which extends about 25–30 days after conception. Its ability to proliferate and actively invade tissue is lost by mid-gestation and its ability to elicit haemorrhage by eroding host blood vessels is lost soon thereafter. There is a suggestion that the trophoblast nuclei may increase in size with age and that cellular degeneration may be accompanied by formation of a symplasma, which is not normally found in the rodent placenta[5]. These results are consistent with the early cessation of trophoblastic invasion and its pattern of ageing and hyalinization *in utero*[6]. Since proliferation and invasion are such prominent features of young transplants into the adult male, it is unlikely that the loss of trophoblastic vigour, and death, are due primarily to a changing or unfavourable endocrine environment.

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PHARMACOLOGY

Serotonin Depletion by Dietary Leucine

Maple syrup disease (branched-chain ketonuria) is an apparently familial disease associated with mental retardation and biochemically characterized by enhanced urinary excretion of branched-chain amino-acids and their products.

Few cases of this disease have been reported[1–4] since its characterization by Menkes, Hurst and Craig in 1954 (ref. 5), and little is known about its biochemical basis beyond suggestive evidence implicating impaired oxidative decarboxylation of branch chain α-keto-acids[6]. Still less is known about the relationship between biochemical features of the disease and the attendant mental retardation.

Mental retardation is a prominent clinical feature both of maple syrup disease and the more familiar phenylketonuria, and attempts have been made, with some success, to establish corresponding biochemical similarities between these diseases. Thus, branched-chain and aromatic amino-acids, and/or their metabolites, inhibit rat brain decarboxylase[7], serotonin and 5-hydroxytryptophan uptake by rat brain slices[8], and, when added as a dietary supplement, reduce liver tryptophan hydroxylase activity[9].

These similarities prompted us to determine whether the brain serotonin-level would be diminished by dietary leucine as it is by dietary phenylalanine[10].

At 17 days of age litter-mate pairs of Sprague–Dawley rats[11], matched for age, weight and sex, were divided into control and experimental groups, housed in individual cages and fed *ad lib.* either a control diet[11] or the control diet supplemented with 5 or 8 per cent by weight of L-leucine[12]. After 30–40 days on the diet, animals were killed by decapitation, the brain removed immediately, sectioned between medulla and spinal cord, rinsed in saline, blotted dry, and analysed for serotonin[13]. Statistical significance was determined using a *t* test for paired differences.

Brain serotonin-levels were diminished by dietary leucine in a manner analogous to that observed using dietary phenylalanine (Table 1). Experimental animals significantly differed from controls in both instances ($P < 0.05$).

Table 1. Depletion of Brain Serotonin by Dietary Leucine

	Body-wt. (g) Mean ± *S.D.*	Brain-wt. (g) Mean ± *S.D.*	Serotonin (μg/g brain) Mean ± *S.D.*
Controls	187 ± 8	1·726 ± 0·048	0·609 ± 0·033
5 per cent leucine	190 ± 13	1·741 ± 0·054	0·518 ± 0·036
8 per cent leucine	170 ± 8	1·626 ± 0·024	0·473 ± 0·010

Growth was impaired somewhat by 8 per cent but not 5 per cent leucine supplementation, although control animals did not significantly differ from those fed the 5 or 8 per cent diets because of the large variance in body-weights. Brain-weights reflected these differences in body-weight—animals on the 5 per cent diet having brains not significantly heavier than controls and animals on the 8 per cent diet having ones significantly lighter. The ratio of brain- to body-weight was essentially the same for all three groups.

Total brain serotonin was 1·084 μg for controls, 0·969 μg for the 5 per cent group and 0·863 μg for the 8 per cent group, again indicating diminished serotonin with increased dietary leucine. Because of the differences in brain weight, however, the controls differed significantly only from the 8 per cent group ($P < 0.001$); the difference from the 5 per cent group reached the 0·1 level of statistical significance. Correcting for body-weight differences by calculating μg serotonin/g brain/kg body-weight, the control value of 3·21 was significantly greater than 2·69 for the 5 per cent group ($P < 0.05$) but not significantly greater than the 2·78 for the 8 per cent group.

Despite these complications resulting from altered growth rates, it seems clear that dietary leucine supplementation lowers brain serotonin. It has been suggested[14] that the decreased brain serotonin in the phenylalanine-fed animals is related to impairment in problem solving ability. The present finding suggests a possible common basis for the mental deficiency accompanying phenylketonuria and maple syrup disease.

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IMMUNOLOGY

Mathematical Model for the Process of Aggregation in Immune Agglutination

Immune haemagglutination is the combination of bivalent antibody molecules with specific antigenic determinants on the surface of red cells and the coincident collision of red cells resulting in the formation of antibody cross-linkages between cells and visible aggregation[1,2]. As a means of characterizing immune haemagglutination, rates of red cell aggregation were examined with the aid of a Coulter electronic cell counter[3]. In the course of this examination it was empirically found that the initial decrease in free cell concentration followed an exponential decay curve. There was no basis, however, for considering aggregation to be a first-order reaction. Subsequent consideration suggested that aggregation involved an orderly sequence which might be expressed mathematically.

The mathematical representation of the rate of red cell aggregation presented in this report was arrived at through several series of experiments in which theoretical and experimental curves were compared. The eventual close approximation achieved between these curves tends to substantiate the validity of the equations from which the theoretical rate curves were derived.

The experimental curves were obtained by a procedure previously described in detail[3]. The rate of reduction in free cell concentration and total number of particles (total of free cells plus aggregates) was followed by means of the 'Model *B*' Coulter electronic cell counter (Coulter Electronics, Hialeah, Florida). Rates were determined under controlled conditions of antiserum concentration, cell concentration, temperature and speed of mechanical mixing. The reaction vessel of 150 ml. capacity was secured in a temperature control jacket, which fits on the stand of the Coulter counter. Agitation was accomplished with a spiral stirrer attached to a constant speed motor, 300 r.p.m. The 100-μ aperture tube had a sampling time of 15 sec. The temperature was maintained at 25° C.

With the double thresholds arrangement of the Coulter counter it was possible to follow experimentally the concentration of several different size aggregates, but because of practical limitations concerned with inhomogeneity in the size of red cells, and complications concerned with coincidence, only the total particle count was followed.

An initial lag period in the rate curve, due to the time required for the primary union of antibody and cells, was eliminated by incubating antiserum and cells without mechanical mixing[3]. At the antiserum and cell concentrations employed in these experiments, a sensitization period between 30 and 80 min was sufficient to eliminate the lag period.

Several immune systems of different specificity were examined in these studies. Human A and B isoantibodies and human red cells of appropriate blood group were used in early experiments. Variation in the degree of fit between theoretical and experimental rate curves obtained with different cell populations suggested a heterogeneity of the red cell population with respect to their agglutinability[4]. It was found that this heterogeneity could be minimized with any of several antigens coupled to tanned red cells[5]. The agglutination obtained with human anti-A sera and purified hog A substance coupled to tanned group O human red cells was most intensively examined.

Immune aggregation can be defined as the change in state from free cells to aggregates due to the random collision of partially antibody-coated (sensitized) red cells. The random collision of sensitized cells leads to a progressive decrease in the number of free cells with a concomitant increase in the number of aggregates of larger size with time. Although the concentration of free cells would decrease throughout the process of aggregation, the concentration of progressively larger size aggregates would consecutively increase and then decrease as aggregation proceeds. The combinations which lead to the formation and subsequent decrease of different size aggregates are shown in Table 1. Based on these considerations a mathematical model for red cell aggregation was progressively developed. The present form of the model will be explained.

Table 1. Combinations which increase and decrease the Concentration of Different Size Aggregates

Aggregate size	Combinations which: increase number of aggregates	decrease number of aggregates
1	0	(1,1) (1,2)....(1,N)
2	(1,1)	(2,1) (2,2)....(2,N)
3	(1,2)	(3,1) (3,2)....(3,N)
4	(1,3) (2,2)	(4,1) (4,2)....(4,N)
5	(1,4) (2,3)	(5,1) (5,2)....(5,N)
6	(1,5) (2,4) (3,3)	(6,1) (6,2)....(6,N)
7	(1,6) (2,5) (3,4)	(7,1) (7,2)....(7,N)
8	(1,7) (2,6) (3,5) (4,4)	(8,1) (8,2)....(8,N)
9	(1,8) (2,7) (3,6) (4,5)	(9,1) (9,2)....(9,N)
10	(1,9) (2,8) (3,7) (4,6) (5,5)	(10,1) .....(10,N)
.		
.		
.		
N	(1,*N*-1).....	(*N*,1).....

It is assumed that there may be aggregates of 1, 2, 3 . . . N cells. The number of aggregates consisting of i cells at time t can be denoted by $X_i(t)$.

To analyse the change in $X_i(t)$ with time, let $A_{ij}(t)$ be the rate at which the number of aggregates of size i combine with aggregates of size j at time t. Notice that if $i=j$, then only $A_{ii}(t)/2$ aggregates of size $2i$ are produced. To make this clear, consider the following two examples. If 5 aggregates of size 3 combine with 5 of size 4, then $A_{34}(t)=5$ and 5 aggregates of size 7 are formed. But if 5 aggregates of size 3 combine with another 5 of size 3, then there are 10 aggregates of size 3 which each combine with an aggregate of size 3, so $A_{33}(t)=10$ whereas only 5 aggregates of size 6 are produced.

The next consideration was those combinations of aggregates which increase the number of aggregates of size i. The combination of an aggregate of size j with one of size k will do so if $j+k=i$. Thus at each time t, $X_i(t)$ is increasing at the rate of the sum of A_{jk} with $j+k=i$. If $j=k$, then the appropriate term is $A_{jj}(t)/2$. In order that each combination be considered only once, the following restriction is added: $j \leqslant k$; thus $A_{12}(t)$ and $A_{21}(t)$ are not both added to $X_3(t)$.

The combinations of aggregates which decrease $X_i(t)$ are all those of the form $A_{ij}(t)$ subject only to the condition that $i+j \leqslant N$.

Putting this information together, the following system of differential equations may be written, where $X'_i(t)$ is the rate change of X_i at time t, that is, $dX_i(t)/dt$.

If i is odd, then:

$$X'_i(t) = \sum_{\substack{j<k \\ j+k=i}} A_{jk}(t) - \sum_{i+j\leqslant N} A_{ij}(t) \quad (1)$$

If i is even, then:

$$X_i(t) = \sum_{\substack{j<k \\ j+k=i}} A_{jk}(t) + A_{i/2\ i/2}(t)/2 - \sum_{i+j\leqslant N} A_{ij}(t) \quad (2)$$

To complete the model, $A_{ij}(t)$ was derived. The rate of combination of aggregates of size i with aggregates of size j should be proportional, according to the law of mass action, to the product of the concentration of the interacting aggregates. Thus, $A_{ij}(t) = C_{ij}\ X_i(t)\ X_j(t)$, where C_{ij} represents the readiness of aggregates of size i to combine with aggregates of size j. It is assumed that C_{ij} is proportional to the product of the average surface area of aggregates of size i and of aggregates of size j, that is $C_{ij} = C\ s_i\ s_j$, where s_i and s_j are the average surface of aggregate of size i and j. We thus have:

$$A_{ij}(t) = C\ s_i\ s_j\ X_i(t)\ X_j(t) \quad (3)$$

Because the shape of the red cell is a non-rigid biconcave disk, a mathematical estimate of the effective surface area of aggregates would be a major undertaking. It was therefore decided to use a physical model consisting of rigid spheres to obtain an approximation of aggregate surface area for small aggregates. Models of different aggregate size, with maximum contact among the spheres, were constructed. The effective surface area of each aggregate was estimated by attempting to fit a free sphere into the aggregate and then tracing around it. The exposed traced areas were cut out and weighed for each model aggregate. Based on the weight of the fragments, the relative effective surface of single spheres and aggregates of sizes between one and nine were calculated (Table 2). For aggregates beyond nine, it was assumed that the aggregates were spherical and the increase in surface was calculated per unit increase in volume. Thus, for sizes greater than nine, surface area was obtained recursively. $s_i = (i/i-1)^{2/3} s_{i-1}$.

Table 2. The Relative Effective Surface Area of Different Size Aggregates (I–IX) based on a Rigid Sphere Model

I	II	III	IV	V	VI	VII	VIII	IX
1·00	1·48	1·81	2·07	2·32	2·59	2·88	3·14	3·41

Having obtained values for the surface area of different size aggregates, C of equation (3) remains as a parameter which varies with the aggregating potential of the sensitized cells. This parameter would include any factor which affects either the quantitative or qualitative characteristics of the antigen-antibody reactions responsible for agglutination[6]. For example, at different antiserum concentrations the red cells are sensitized to different degrees, altering the rate at which antibody cross-linkages are formed between cells and, therefore, the rate of aggregation. A fit of such different rate curves could be achieved by substituting an appropriate value for C.

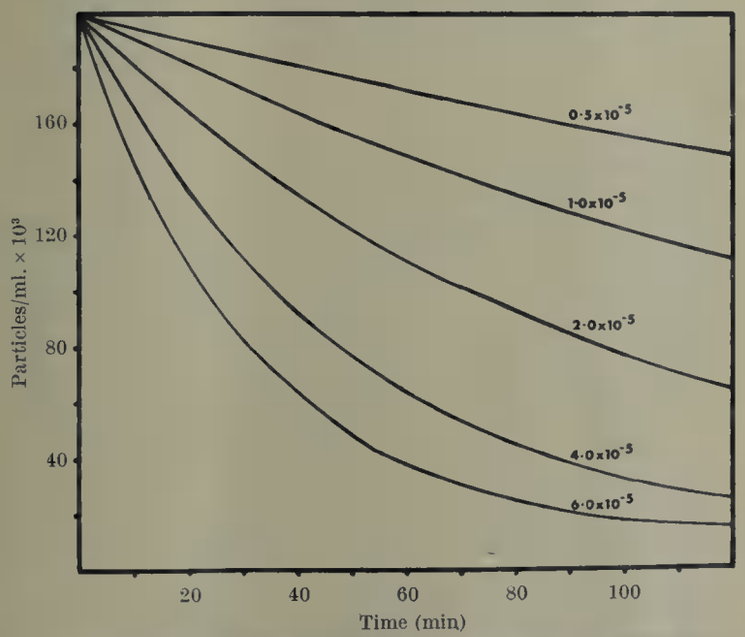

Fig. 1. A plot of curves representing the concentration of particles with time, substituting different values for C (aggregating potential of cells)

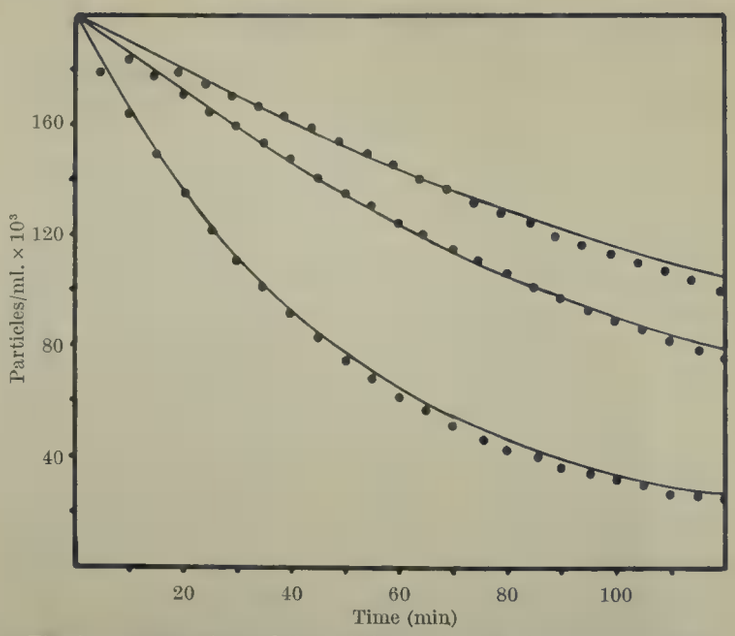

Fig. 2. A comparison of three different theoretical and experimental curves obtained with human anti-A sera and hog A substance coupled to tanned human O cells. The theoretical curves are represented by solid lines and the experimental curves indicated by points. Values of C: $1{\cdot}1\times10^{-5}$, $1{\cdot}6\times10^{-5}$ and $4{\cdot}0\times10^{-5}$

The computations required to solve the equations for a theoretical rate curve were performed on an IBM '7094' computer and were obtained by the recursive formula: $X_i(t+1) = X_i(t) + X'_i(t)$, t being in minutes with the boundary conditions that all $X_i(0) = 0$ except for X_1 which equals the initial number of free cells. In these computations, aggregates up to 100 cells in size were allowed. Several theoretical curves of total particle count with different values of C are shown in Fig. 1. At low values of C a nearly linear rate is obtained. With a progressive increase in the value of C there is a decreasing effect on the rate curve. Theoretical curves and experimental curves obtained with different concentrations of antiserum are compared in Fig. 2. It can be seen that the mathematical model does give a reasonably close approximation to the rates of aggregation obtained experimentally.

According to the foregoing considerations, the process of immune aggregation is pictured as a sequence of second-order reactions which can be mathematically expressed as *a system of second-degree first-order differential equations with constant coefficients*.

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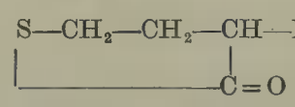
RADIOBIOLOGY

Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone

Some time ago we discussed the formation of radicals in irradiated samples of methionine[1] and bovine serum albumin[2]. In the case of methionine, in which the bivalent sulphur is present in the form of a thioether, X-irradiation forms radicals of the alkyl-type, but ultra-violet irradiation (254 mμ) localizes them at the sulphur atom. Moreover, ultra-violet light is able to quench the alkyl-type radicals. In the case of bovine serum albumin, X-irradiation as well as ultra-violet light forms a complex electron spin resonance spectrum consisting of a glycylglycine- and a sulphur-radical[3]. The portion of the sulphur radical part becomes bigger after ultra-violet irradiation. By ultra-violet irradiation of longer wave-length ($\lambda > 300$ mμ) the X-ray conditioned radical state at the carbon atom is transferred to the SS- or SH-groups of the albumin molecule. In the following investigations we controlled the behaviour of the bivalent sulphur in the form of a thiolactone ring compound, that is, a thioester, homocysteinethiolactone (HCT):



Polycrystalline, *d,l*-homocysteinethiolactone (HCT)-HCl (Degussa, Konstanz/Germany) with a bulk volume of about 220 cm³/100 g was sealed under vacuum ($< 10^{-3}$ torr) and irradiated at room temperature. The electron spin resonance measurements were carried out under the same conditions as previously described[1]. The X-irradiation was carried out with a 200 kV source (half value layer: 0·64 mm copper, dose-rate 920 r./min, dose 100 kr.). The irradiations with ultra-violet were performed with a mercury low-pressure lamp (*NN* 15/44, Quarzlampengesellschaft, Hanau, Germany) which emits mainly in the 254 mμ line. The intensity of the ultra-violet irradiation at the sample tube amounted to 9×10^3 ergs cm^{-2} sec^{-1}. The other technical details have been reported in a former publication[1].

After X-irradiation a radical yield of 10·8/100 eV is obtained. The electron spin resonance signal of the X-irradiated HCT is demonstrated in Fig. 1 (solid line). Though it is not possible to differentiate the obtained spectrum of the polycrystalline material, it is possible to ascertain that there exists no sulphur radical state. The different behaviour of saturation effects of the micro-wave energy on the different parts of the spectrum includes at least two different radicals. We suppose that the unpaired electrons are localized at a carbon and/or nitrogen atom of the molecule. The radicals are very stable not only in vacuum but also in air. In vacuum and at room temperature the decay of the radical concentration is only about 1 per cent/h.

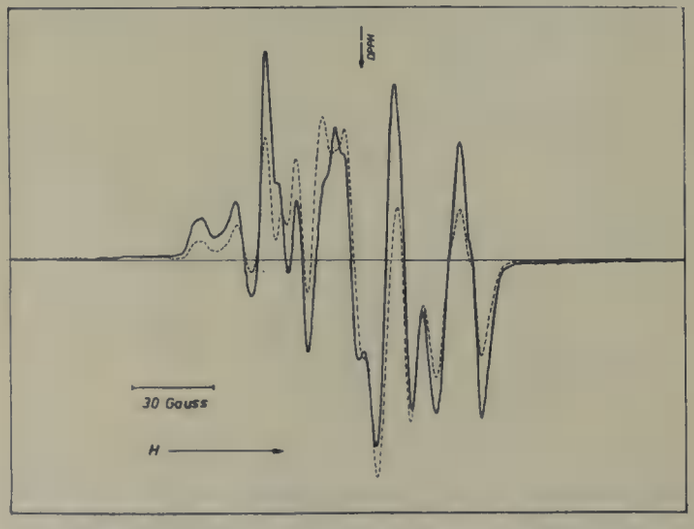


Fig. 1. Electron spin resonance spectra of irradiated homocysteinethiolactone. The spectra represent the first derivatives of the actual absorption curves. ——, 100 kr; – – –, 100 kr + 7 h ultra-violet ($\lambda = 254$ mμ)

In contrast to methionine and bovine serum albumin, ultra-violet light of 254 mμ has no influence on the X-ray signal of homocysteinethiolactone. The dotted line in Fig. 1 shows the change of the X-ray spectrum after an ultra-violet irradiation of 7 h. This dotted line spectrum demonstrates clearly that only one part of the former spectrum is influenced by quenching. The same change is obtained by warming up the X-irradiated sample of HCT. In comparison with the solid line spectrum (X-ray alone) the radical concentration was found to be diminished after ultra-violet irradiation to nearly 88 per cent. It is very interesting that no sulphur radical is detectable although the lactone ring is opened by the ultra-violet irradiation. The latter process is certain because hydrogen sulphide gas is liberated. Perhaps the yield is not big enough to detect the sulphur radicals by the sensitivity of the electron spin resonance spectrometer.

If HCT is irradiated only with ultra-violet light of the wave-length of 254 mμ for 8 h, a radical concentration of only 5 per cent is obtained corresponding to that of an X-irradiation with 100 kr. This ultra-violet signal is not decomposed, it might be a quintet and centred at $g = 2{\cdot}0046$. In contrast to sulphydryl-, disulphid- and thioether-compounds, there is no sulphur radical to be found for the thiolactone.

The results we obtained from bivalent sulphur containing compounds demonstrate quite clearly that not only the primary formation of sulphur radicals but also the transfer of the radical state to the sulphur atom depends on the chemical binding of the bivalent sulphur. In our next publication we hope to describe the influence the whole molecule has on the character of the sulphur radical.

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Use of Technetium–99m in Hepatic Scintillography

Harper *et al.*[1] have recently pointed out the excellent possibilities of technetium–99m in medical scintillography.

Tc–99m is a nucleid with a period of semidisintegration time of 6 h, emitting γ-radiation of 140 keV, obtained by acid elution of molybdenum-99 adsorbed on an aluminium column. It is obtained as a pertechnetiate (TcO_4^-), which has a behaviour similar to the I^- ion in the body. We have used it with very good results in thyroid scintillography[2,3]. Harper has also used this compound combined with a colloid which is retained by the reticulo-endothelium in liver scintillography[1]. The Production Department of the Comisión Nacional de Energía Atómica, Argentina[4], has obtained a colloid of antimonium sulphide which has a molecular size of 100 to 400 mμ, an adequate take-up of ^{99m}Tc, and appears to be very stable. We have been able to obtain hepatic pictures of excellent resolution by intravenous injection of this substance (Fig. 1). Its concentration in the liver allows us to perform tracing only 10 min after injection and its activity is still at adequate levels after 180 min.

After 24 h the activity eliminated by urine was 10–15 per cent. We have used activities of 0·4–1·0 mc.

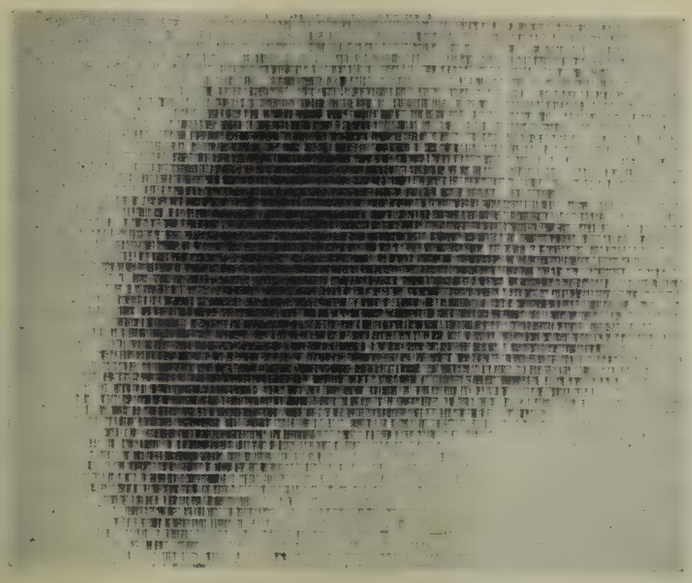

Fig. 1. Normal liver scanning (approximate dose 0·8 mc.) ^{99m}Tc-labelled colloid

which are much smaller than those used by Harper *et al.* The equipment used was a 'PHO-DOT' (Nuclear Chicago). The pictures obtained could be compared satisfactorily with those obtained with rose bengal iodine-131 colloid albumin, the body radiation being several thousand times smaller with ^{99m}Tc.

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BIOLOGY

Multiple Thymus Grafts in Aged Mice

One characteristic of the thymus which has not yet been explained completely is the weight loss (primarily lymphoid depletion) of the organ with increasing age. This process, known as thymus involution, is most marked immediately after puberty, but thymus involution continues, albeit at a slower rate, throughout subsequent life.

The recent data of Harris *et al.*[1] from parabiotic mice and Miller[2] and Metcalf and Wakonig-Vaartaja[3] from thymus grafts have made it evident that the lymphoid population of the thymus is replaced continuously during life by precursor cells seeding in the organ from the blood. These precursor cells then proliferate to build up a large new population of primitive and mature thymic lymphocytes. The most probable source of these thymus-seeding precursor cells is the bone marrow.

Analysis of the growth patterns of thymus grafts has suggested that the growth of the lymphoid elements of the thymus is regulated by the non-lymphoid elements of the organ—the specialized thymic epithelial and reticulum cells[4]. The strength of the proliferative stimulus exerted by these elements appears to decrease with advancing age and, if so, this could be the basis for the decrease in size of the organ with advancing age. However, in view of the continuous replacement of the lymphoid population of the organ throughout life by precursor cells of extrathymic origin, another possible basis for the decline in size of the organ is that, with advancing age, the supply of thymus-seeding precursor cells becomes restricted, leading to a limitation in the total amount of thymic lymphoid tissue which can be built up, and sustained, by the proliferative activity of these cells.

To investigate the possibility that the supply of thymus-seeding lymphoid precursor cells may be limited in old animals, use was made of an earlier observation that when more than one thymus is grafted to a young recipient, each graft behaves autonomously, achieving the same size it would if grafted alone to a normal or even a thymectomized recipient[5]. This allows the possibility of growing unlimited amounts of thymus tissue in the same animal and thereby of testing whether the supply of thymus-seeding cells ever becomes limiting for the growth of such grafts—particularly in very old animals. In the present investigation, 20-month-old *C57 BL* mice were each grafted subcutaneously with 12 or 24 whole 1-day-old *C57 BL* thymus glands. One month after grafting the weights of thymus grafts and host lymphoid organs were determined. As controls, *C57 BL* thymus glands were grafted to young adult *C57 BL* mice (either 1, 12, 24 or 48 per recipient) and these grafts were also examined one month after grafting. The results obtained are summarized in Table 1. In agreement with previous investigations[5], in the young hosts, a linear relationship was observed between the number of thymus glands grafted and the total weight of thymus graft tissue recovered. In neither type of host did the multiple thymus grafts significantly affect the weights or the histological appearance of the host thymus or other lymphoid organs. It is evident that the total mass of thymus graft tissue achieved in 4 weeks in the old recipients was somewhat less than for the corresponding number of grafts in young adult recipients. However, the mass of thymus graft tissue achieved far exceeded that of the maximum size of the normal *C57 BL* thymus in young mice (60 mg). Histologically no difference between grafts in young and old hosts could be detected in the morphology, extensiveness and mitotic activity of the cortical lymphoid regions of the thymus graft tissue.

Table 1. Thymus Graft Weights attained by Multiple Whole *C57 BL* Thymus Grafts in Young and Old *C57 BL* Mice

Age of recipient at grafting	No. of thymus glands grafted per recipient*	No. of recipients	Mean host thymus weight (mg)	Mean weight of individual thymus grafts (mg)	Mean total thymus graft weight per recipient (mg)
2 months	1	28	47	24	24
	12	41	42	22	262
	24	2	48	25	609
	48	2	36	24	1,160
20 months	12	18	16	16	185
	24	2	15	19	467

* Mean weight of each donor thymus gland, 3 mg.

Since the lymphoid population of thymus grafts is derived from host cells, it is obvious from the present experiments that, although there may be some restriction in the supply of thymus-seeding precursor cells in old age, the available supply is quite capable of supplying thymus-seeding precursor cells for a mass of thymus tissue far exceeding that of even the pre-involutional thymus. Therefore it may be concluded that no significant limitation in the supply of thymus-seeding precursor cells exists in older mice and such a factor cannot be responsible for the decrease in size of the thymus with advancing age. Recently, Makinodan and Peterson[6] have demonstrated that in old mice the number of cells in the spleen capable of being stimulated by sheep red cells does decline in old age, and we have obtained similar results in *C57 BL* mice. The cell population in the spleen is replaced continuously by cells entering from the blood in essentially the same manner as is that in the adult thymus. It appears therefore that although the supply of some spleen-seeding cells may become restricted in old age, there is no significant

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TE31

presence of fructose-1-phosphate in storage tissue of carrot (*Daucus carota* L.) and white turnip (*Brassica rapa* ssp. *rapa*).

Disks (1 mm × 10 mm) of the tissues were aerated at 25° C for 17 h in distilled water and then in 0·1 mM $KH_2{}^{32}PO_4$ for a further 24 h. The tissue was rinsed and extracted successively with boiling 80 per cent ethanol, 20 per cent ethanol and water. The phosphate esters in the combined extracts were fractionated by ion-exchange resin column chromatography using a technique[5] similar to that of Bartlett[6]. The hexose monophosphates, free from hexose diphosphates, were located by their radioactivity, by reaction with anthrone reagent (for hexoses)[7] and by a slightly modified cysteine-carbazole reaction (for fructose)[6]. Fractions containing mainly fructose phosphates were subjected to one-dimensional paper chromatography in each of two solvents. Solvent 1 was ethyl acetate–glacial acetic acid–water, 3 : 3 : 1 by volume[8], and was used with Whatman No. 1 paper. Solvent 2 was *tert.*-butyl alcohol–0·1 N hydrochloric acid, 80:20, v/v (ref. 9), and was used with Whatman No. 4 paper. These solvents separated glucose-1- and glucose-6-phosphates from fructose-1- and fructose-6-phosphates and also gave a partial separation of the two fructose phosphates. Although the separation of fructose phosphates on paper chromatograms was not complete, it was sufficient to enable the two compounds to be distinguished by their characteristic ultra-violet fluorescent reactions as described below.

These procedures revealed the presence of a compound in carrot and turnip extracts which behaved similarly to authentic fructose-1-phosphate. The results in Table 1 compare the P constants:

$$\left\{ \frac{\text{distance travelled by compound}}{\text{distance travelled by orthophosphate}} \times 100 \right\}$$

of the extracted compounds with the P constants of authentic fructose-1- and fructose-6-phosphates and those of other sugar phosphates which could have been components of the eluates obtained from ion-exchange resin column chromatography.

Table 1. P Constants and Ultra-violet Fluorescent Reaction with Glycerol/HCl/Acetone Reagent of some Carbohydrate Phosphates compared with Compounds isolated from Carrot and White Turnip

	P constants		Ultra-violet fluorescent reactions in each solvent
	Solvent 1	Solvent 2	
F-1-P	63	73	yellow
F-6-P	66	81	red-brown
G-1-P	52	67	none
G-6-P	47	67	none
R-5-P	75	92	none
P_i	100	100	none
Carrot	62	75	yellow
	67	82	red-brown
Turnip	62	74	yellow
	67	82	red-brown

Evidence that the compound obtained from carrot and turnip was fructose-1-phosphate was obtained by a modification of the technique of Steinitz[9]. Under our conditions in which the reagent was sprayed onto the chromatogram, authentic fructose-1-phosphate gave an intense yellow fluorescence and authentic fructose-6-phosphate a dull, red-brown fluorescence which changed to yellow on heating for a further 3–4 min. The ultra-violet fluorescent reactions of the isolated compounds obtained under a 'Chromatolite' (Hanovia Lamps, Slough, England) were compared with those of authentic samples of fructose phosphates and other sugar phosphates likely to be present in the column eluate fractions (Table 1). Subsequent development of the chromatograms with the reagent of Hanes and Isherwood[10], as modified by Bandurski and Axelrod[11], confirmed that the isolated compounds contained phosphate.

Additional evidence that the plant extracts contained fructose-1-phosphate was provided by the demonstration that column eluate fractions, suspected of containing fructose-1-phosphate, contained a substrate for an aldolase preparation isolated from rat liver by the method of Leuthardt and Wolf[12]. This preparation, which is known to cleave both fructose-1,6-diphosphate and fructose-1-phosphate, was used to assay the column eluate fractions for fructose-1-phosphate by linking the production of dihydroxyacetone phosphate to the oxidation of $NADH_2$ on the addition of purified α-glycerophosphate dehydrogenase. Values of 1·95 and 2·11 μmoles of fructose-1-phosphate/100 g fresh weight were obtained for carrot and turnip tissue respectively.

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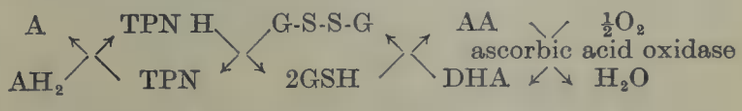
Some Postulated Relationships among Autumn Phenomena in Woody Plants

Most research on winter hardiness (cold acclimation) of plants has been concerned with changes in amounts of organic compounds such as sugars, amino-acids and proteins. These autumnal alterations in organic constituents of the cell can only be brought about by changes in enzymes. The question as to what initial factor causes this change in the metabolism is still not solved. It is tempting to think of a hormone action which could influence a great many enzyme systems[1], especially since cold acclimation and de-acclimation are closely associated with the growth-regulator controlled phenomenon of growth cessation in autumn, and growth resumption in spring. Recently, Levitt[2] has suggested that protein and glutathione sulphydryls bear a direct relationship to the state of winter hardiness of plants. Sulphydryl groups in cells are essential for the three-dimensional structure of cell proteins[3] and for cell growth[4]. Glutathione is also an essential part of the ascorbic acid oxidase system which is involved in growth processes, that is, ascorbic acid oxidizing capacity is low in growing plants and high in dormant plants. Marré and Arrigoni[5] presented evidence that auxins inhibited the ascorbic acid oxidase and thereby increased the ratio of reduced to oxidized glutathione. A postulated relationship between ascorbic acid oxidase and glutathione can be represented as follows:



AH_2, metabolic substrate; G-S-S-G, oxidized glutathione; GSH, reduced glutathione; AA, ascorbic acid; DH,A dehydroascorbic acid.

It has also been found[6] that there is a shift in wheat caryopses from the cytochrome oxidase system to the ascorbic acid oxidase system after cold treatment. An increase in glutathione oxidizing activity, along with an apparent rise in ascorbic acid oxidizing activity, was observed in cold acclimating plants[7]. This high rate of

ascorbic acid oxidizing activity is contrary to the low capacity in vigorously growing tissue and thus must be due to an absence or inhibition of growth-promoting substances in cold treated tissue.

It has been reported by several investigators[8,9] that the biosynthesis of growth promoters in woody plants levels off during the growing season, and that the concentration of growth inhibitors increases toward autumn. It has also been reported that there was a significant increase of tryptophan in dormant tissue after low-temperature treatment[10]. As tryptophan is considered to be the precursor of indolyl-3-acetic acid[11] its accumulation would suggest an arrest of auxin synthesis. An actual decrease of auxin has been reported to occur in cold-acclimated plants[12].

Changes in growth regulators may influence many processes. It has been reported that they are able to affect the bioelectric potential of the plant[13] and thereby may change the cell membrane permeability[14] which is a prerequisite for the cold acclimation process[15]. They may also affect the properties of proteins[16] as well as the enzyme action already mentioned.

Growth inhibitors may affect the hardiness process as the synthetic growth inhibitor maleic hydrazide does[17], via the sulphydryl groups of the mitotic cell[18]. Several natural growth inhibitors have been reported to occur[19]; many are flavonoid-like structures[20,21]; others, such as β-inhibitor[22], have only been tentatively identified[23]. Flavonoids have been known to influence plant metabolism in several ways[24].

The phenomena in red-osier dogwood during autumn occur in the following order[25]: irreversible rest inducement, cold acclimation, and loss of rest. The sequence of this process could be in these steps. An inducement of rest is brought about by some kind of phytochrome system[26]. At this time, a hormone is produced which causes an increase in RNA content[27] and a subsequent increase of water-soluble proteins. Although the nature of these water-soluble proteins is obscure, some of them may represent an increase of enzymes which are responsible for the increase of carbohydrates and other organic products in cold acclimating plants.

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Freeze-fixation, a New Method for Electron Microscopy

Freeze-fixation implies rapid freezing of small specimens followed by vapour fixation at a low temperature. This method differs from freeze-drying by not removing the water of the specimen before fixation, and from the freeze-substitution by not replacing the water during fixation. All these three methods work with frozen tissue, but since the mode of fixation varies the ultrastructure of the cellular constituents also varies. Therefore, further information of the cellular constituents might be obtained.

For freezing, small specimens are rapidly frozen in liquid nitrogen according to the usual technique for freeze-drying. The frozen specimens are collected in a small weighing bottle with grounded stopper. The bottle contains a substance suitable for vapour fixation and serves as a fixation chamber after freezing.

For fixation, the bottle containing specimens and fixation substance, and filled with liquid nitrogen, is placed in dry ice. At this temperature, the liquid nitrogen evaporates. The bottle is then firmly closed with its stopper and left in a freezer for 2–3 weeks for vapour fixation. The only fixation substance as yet tried in the experiment is crystalline osmium tetroxide. After the period in dry ice, the specimens are either left in the bottle for further fixation at room temperature or brought to the appropriate solution of the embedding chemicals used. In the present experiments, plastic embedding has been started in 70 per cent ethanol for the 'Epon' procedure or 25 per cent component '*A*' of the 'Durcopan' procedure.

The main drawbacks of freeze-fixation are the low vapour pressure of the fixation substance at −30° C and the slow penetration of the fixative into the frozen tissue. The specimens therefore must be small, preferably single cells. The morphological quality of the tissue is inferior to that obtained after freeze-drying. Thus, the several artefacts impede investigations of the whole cell, but they do permit analysis of, for example, the cell membrane.

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MICROBIOLOGY

Endogenous Bud Formation in the Hypotrichida

As a general rule, ciliated protozoans reproduce asexually by transverse binary fission. Suctoria, however, characteristically produce unequal endogenous buds which develop into migratory ciliated larval forms. Endogenous budding has not been reported in the remaining subclasses of the Ciliatea. Observations made by Kent suggest that one member of the Hypotrichida has a larval stage in its life-cycle[1]. He observed the development of an embryonic hypotrich, which closely resembled *Glaucoma margaritaceum* Ehr., into *Aspidisca costata* Ehr. In the same treatise Kent considers the holotrich genera *Glaucoma* and *Microthorax* to be embryonic or transitional phases in the life-cycles of higher hypotrichous forms. No other reference to such life-cycles in the Hypotrichida have been found.

This communication presents preliminary observations made on monoxenic clonal cultures of the hypotrichous ciliate *Histriculus vorax* Corliss. *Bacillus cereus* was used as the sole food organism, and the ciliate culture was originally separated from activated sludge.

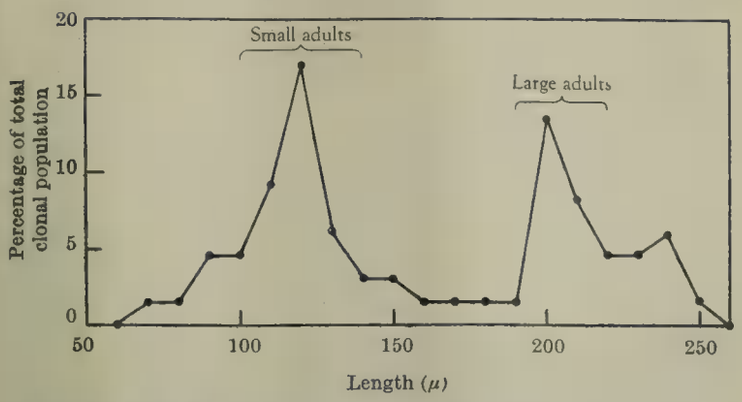

Fig. 1. Size frequency curve showing the presence of two distinct adult cell sizes within a well-fed clonal population of *Histriculus vorax*

Great variations in size have been noted in clonal populations, but 70 per cent of the cells in such populations fall within two definite ranges of size (Fig. 1). Large adult cells (190–220μ long, 80–100μ wide) are bluntly shaped, whereas small adult cells (100–140μ long, 50–60μ wide) are streamlined in their appearance. Both adult forms are capable of normal transverse division resulting in the production of two daughter cells the dimensions of which, after a short period of growth, resemble those of the mother-cell. The large adult cells form 20–30 per cent of a well-fed clonal population, but under conditions of starvation they are absent.

Observation has shown that the large adult cells (Fig. 2) develop endogenous bi-macronucleate buds (20–40μ diameter) which, when mature, are extruded through the cell wall. After extrusion, the mother-cell may either then produce more buds endogenously or divide transversely. Newly deposited buds may either develop directly into a cirriated larval form or, if conditions are unfavourable, the bud will encyst.

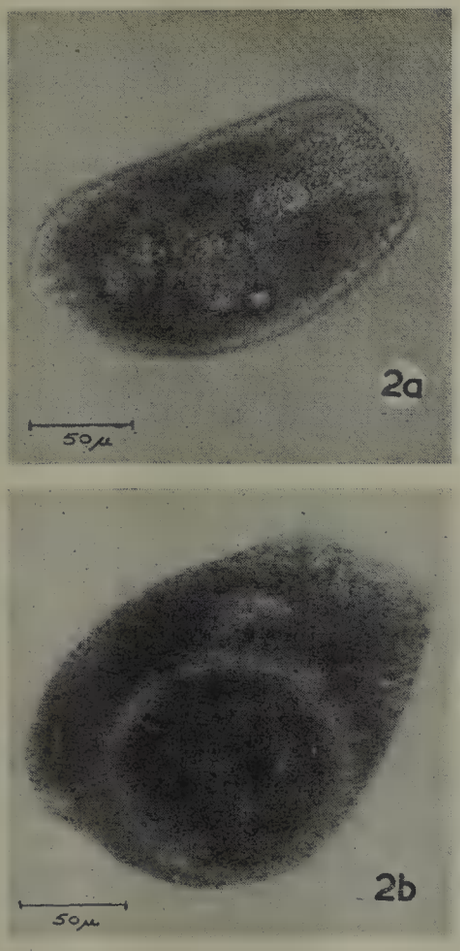

Fig. 2. Formation of endogenous buds in *Histriculus vorax* Corliss. *a*, Large adult containing two developing endogenous buds; *b*, large adult mother-cell containing a mature endogenous bud

Isolated encysted buds have been excysted by placing them into fresh culture media. Excystment takes approximately 48 h to complete at room temperatures, and terminates when a non-trophic migratory larval form, which bears only caudal and frontal cirri, emerges (Fig. 3). Under favourable conditions newly deposited buds develop directly into larval forms which cannot be distinguished from the embryos which emerge from encysted buds. The non-trophic embryos rapidly develop feeding membranelles and a full adult complement of cirri. The trophic larval stage grows and quickly develops into a small adult capable of transverse binary fission.

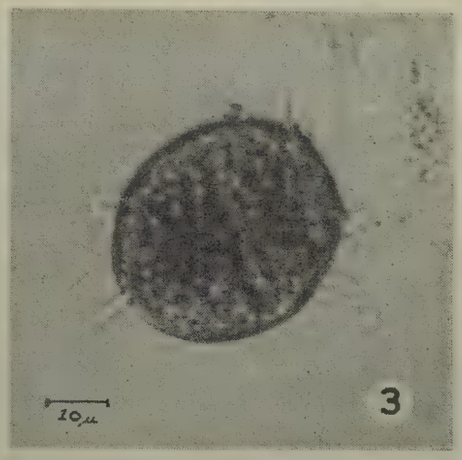

Fig. 3. Non-trophic embryonic stage of *Histriculus vorax* Corliss

This work was supported by the Department of Scientific and Industrial Research while I was holding a research fellowship.

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Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative Humidity

In a recent article[1] describing the effects of humidity on the inactivation of micro-organisms, the results of some preliminary experiments with X-rays were presented. The present communication reports an extension of this work in which the effect of atmospheric oxygen in modifying the radiation sensitivity at various humidities was investigated in addition to the protective effect of inositol. Inositol was of particular interest because of its apparent ability to replace water in biologically important macromolecules[2] and in this way to protect cells against inactivation by drying, ultra-violet and X-rays[1,3].

The effect of inositol appears to be similar to that of glycerol, which has been the subject of a number of recent investigations[4–10]. The role of water in the radiation inactivation of bacterial spores and vegetative bacteria has been extensively studied by Powers and his colleagues[6,7,11,12].

For the experiments reported here, a sub-culture of *Serratia marcescens* was prepared by incubation in heart infusion broth for 48 h. Cells from the culture were centrifuged, washed, and re-suspended in either distilled water or a 5 per cent (0·3 M) solution of meso-inositol. One ml. samples from the foregoing suspensions containing about 5×10^9 cells were deposited on 'Millipore' filters. The filters were placed in an irradiation chamber through which air or nitrogen of the desired relative humidity could be passed. The relative humidity of the gas which had passed over the filters was measured with a membrane-type meter ('Serdex'). The meter reading was dependent not

only on the relative humidity of the gas entering the irradiation chamber, but also on the degree to which the cells had come into equilibrium with the relative humidity of this gas. For experiments with a nitrogen atmosphere, commercial nitrogen (oxygen concentration less than 70 parts per million) was passed through the irradiation chamber for at least 1 h before beginning the irradiations.

After equilibration, cells were irradiated with unfiltered 200 kVp. X-rays (half-value thickness, 8 mm aluminium) at an exposure rate of 790 r./min. The irradiations were carried out at room temperature (22° C—25° C). In some experiments, cells were irradiated in suspension after aeration of the suspension by bubbling air through it. After irradiation, the cells were plated on heart infusion agar. Visible colonies were scored after 24 h incubation at 35° C.

The survival curves obtained under all experimental conditions appeared to be exponential and were fitted by an expression of the form $N/N_0 = \exp(-kD)$, where N and N_0 are the number of colony-producing cells in the irradiated samples and the unirradiated control, respectively, D is the absorbed dose and k is an inactivation constant, which was used as an index of radiation sensitivity under the various conditions. In the control samples it was found that 30–50 per cent of the cells deposited on the filter would later produce colonies even after storage at a relative humidity as low as 25 per cent for several hours.

Values obtained for the inactivation constant for *Serratia marcescens* irradiated in air are shown in Fig. 1. The relative humidity values on the abscissa scale refer to the atmosphere above the cells. The circles and upper curve give values for k obtained for cells deposited from a water suspension, the triangles and the lower curve for cells deposited from a 5 per cent inositol solution. It may be seen that inositol has a protective effect at all relative humidities and that this protection is nearly constant at humidities below 70 per cent. This is in contrast to the results for ultra-violet irradiation of cells in aerosols in which a marked increase in the protective effect of inositol with decreasing relative humidity was found[1]. The abrupt decrease in sensitivity at a relative humidity of about 80 per cent in both the curves shown in Fig. 1 is believed to be due to the removal of 'free' water from the cell and therefore to a decrease in the 'indirect' effect produced by the radiolysis of water.

This decrease is in contrast to the results of Tallentire and Powers[12] for the bacterial spores and those of Webb[6] for vegetative cells of *Staphylococcus aureus*. These workers observe an increase in sensitivity with decreasing water vapour pressure if oxygen is present during and after the irradiation. The difference may be due to differences in the procedure by which the cells are dried.

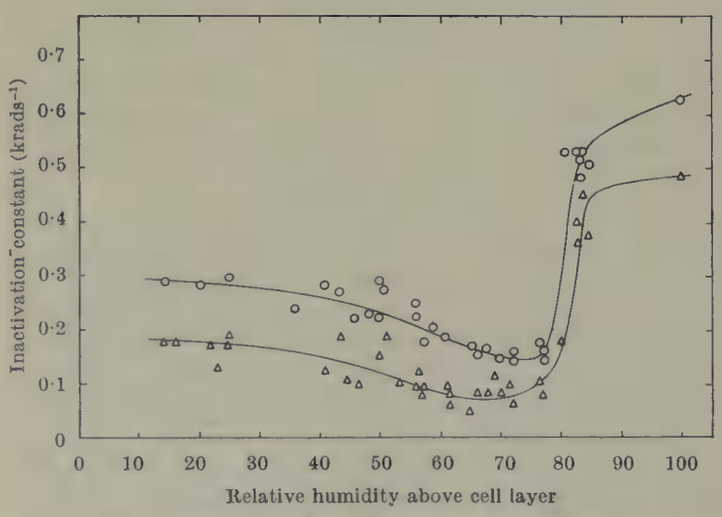

Fig. 1. X-ray sensitivity of *Serratia marcescens* cells irradiated under an atmosphere of air, having previously been suspended in water (○) or in 5 per cent inositol (△)

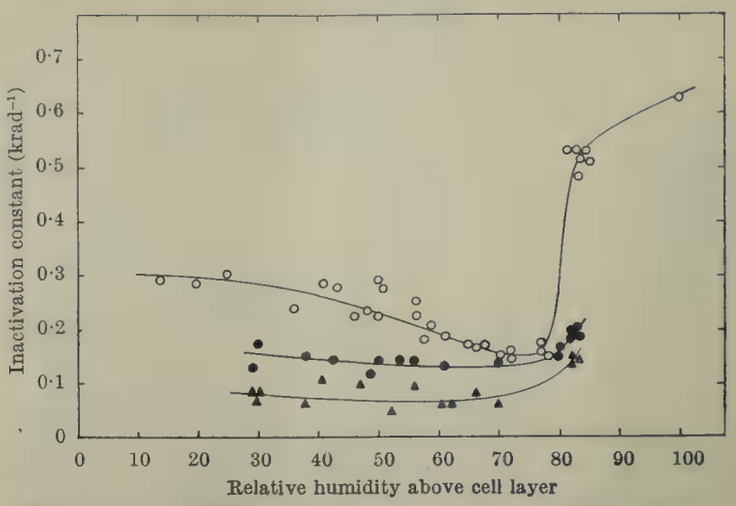

Fig. 2. X-ray sensitivity of *Serratia marcescens* under various conditions indicated. ○, Water–air; ●, water–nitrogen; ▲, inositol–nitrogen

In Fig. 1 the shape of the curves below 70 per cent is consistent with a model in which the inactivation constant is assumed to have two components one of which, k_0, is independent of water in the cell and the other, k_1, is proportional to the amount of water which has been removed by drying, taking the amount of water at various relative humidities from data measured by Webb[3]. The increase in k_1 with decreasing humidity does not appear to correspond directly to the much greater increase observed by Powers and his colleagues for bacteria and spores irradiated and stored in the presence of oxygen[6,12].

The measured inactivation constants for cells irradiated under a nitrogen atmosphere are shown in Fig. 2. The water–air curve from Fig. 1 has been included in Fig. 2 to facilitate comparison. For cells deposited from a water suspension and irradiated at high humidities (above 80 per cent) there is a marked decrease in sensitivity when air is replaced by nitrogen. This 'oxygen effect' nearly disappears at a relative humidity of about 70 per cent, suggesting that the damage due to the 'indirect' effect, postulated here, requires the presence of oxygen. Below 70 per cent the inactivation constant for cells irradiated in air increases with decreasing relative humidity whereas that for cells irradiated in nitrogen appears to remain nearly constant. It therefore appears that the inactivation described by k_1, which we have associated with the amount of water in the cell, requires the presence of oxygen. The humidity-independent component, k_0, appears also to be independent of oxygen and therefore to correspond to the Class I damage described by Powers and his colleagues[6,11,12].

The triangles and the lowest curve in Fig. 2 give the inactivation constants for cells deposited from an inositol suspension and irradiated in nitrogen. These points fall considerably below those for cells deposited from a water suspension and irradiated in nitrogen. The fact that there is a considerable protective effect in the absence of oxygen indicates that the action of inositol is not simply in interfering with oxygen concentration in the cell. Below 70 per cent relative humidity the inactivation constant for inositol-treated cells irradiated in nitrogen is nearly independent of relative humidity and has a value approximately equal to the minimum value for inositol-treated cells irradiated in air. It therefore appears that at the lower humidities there are two kinds of radiation damage both in inositol-treated cells and in unprotected cells, with the humidity-dependent component in each case requiring the presence of oxygen.

These experiments indicate that, at the lower humidities, inositol exerts most of its protective effect by reducing the oxygen-independent component of the radiation damage. This is in contrast to the observed protection by glycerol of cells in suspension which is considerably greater in the presence of oxygen than in its absence[7,9,10].

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VIROLOGY

Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis Virus

The influence of deuterium oxide (D_2O) on virus replication in tissue culture cells is variable. When poliomyelitis virus (a small, RNA virus) is grown in 20–30 per cent D_2O its proliferation is enhanced[1–3], whereas simian virus 40 (a DNA virus) does not replicate as well in deuterated medium as it does in protonated medium[4]. In an attempt to ascertain whether the size of a virus or its nucleic acid composition may be correlated with the D_2O effect, we have examined the effects on the replication of western equine encephalitis (WEE), a relatively large, RNA-containing virus.

The influence of 25 per cent D_2O on the replication of two mutants of WEE was studied. The two mutants are distinguished by the size of the plaques which they yield when grown on chick embryo fibroblasts. The method of growing the virus was essentially that of Wecker and Schonne[5]. Fibroblasts of ten-day-old chick embryos were cultivated in 100-mm Petri dishes in a growth medium consisting of Eagle's medium in Earle's balanced salt solution and 10 per cent calf serum. The WEE virus was diluted in Hanks's salt solution and 0·2 ml. of the diluted WEE was added to each monolayer. Virus absorption was permitted to proceed for 1 h at 37° C, then the cells were overlaid with nutrient agar (12 ml. of 1 per cent agar in Eagle–Earle's medium containing 10 per cent calf serum). Half the fibroblast monolayers were overlaid with nutrient agar made up in medium containing 25 per cent D_2O. After 3 days of incubation in an atmosphere of 4 per cent carbon dioxide in air, the plaques caused by the WEE virus were counted and measured. The results of two experiments are presented in Table 1. In every case plaque size was smaller in the D_2O medium. The observed differences are highly significant ($P < 0{\cdot}001$).

The data presented here indicate that the nucleic acid moiety of a virus does not necessarily determine the nature of the viral growth response in D_2O. To further elucidate this point experiments involving a small RNA virus (Mengo) and a DNA virus (adenovirus) are in progress.

Table 1. Influence of 25 per cent D_2O on Growth of Western Equine Encephalitis (WEE) on Chick Embryo Fibroblasts

Exp. No.	Plaque mutant	Medium	No. plaques measured	Plaque size (mm) (avg.)
1	Large	H_2O	19	4·4 ± 0·14*
		D_2O	24	3·2 ± 0·16
	Small	H_2O	24	2·6 ± 0·05
		D_2O	20	2·0 ± 0·04
2	Large	H_2O	21	4·7 ± 0·09
		D_2O	24	3·7 ± 0·14
	Small	H_2O	†	2–2·5†
		D_2O	†	1–1·5†

* Standard error.
† Not quantitated.

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CYTOLOGY

Barr Bodies in the Mouse

Up to the present, sex chromatin has not been identified in the nuclei of mouse cells. This is probably due to the fact that such nuclei contain multiple chromocentres, of varying size and number, the presence of which tends to mask the appearance of a sex-specific chromatin mass in females. Moore and Barr[1], in a systematic survey of the nerve cell nuclei of many different mammals, were unable to demonstrate sexual dimorphism in those of the mouse. Later, Hay[2], examining twenty non-nervous tissues of the mouse, could not identify sex chromatin in any of them. Himrichsen and Gothe[3] analysed the multiple chromocentres of murine Purkinje cells, organizing them into eleven arbitrary patterns in accord with their sizes and distribution. Among these patterns there were two configurations with respect to which male and female mice seemed to differ, but neither of these was that of the single nucleolar satellite common to the neurones of female mammals.

A search for peripheral sex chromatin masses—Barr bodies—was made in nuclei of fibroblasts which had grown out from small fragments of mouse peritoneum explanted on to cover slips. This method provided a population of young cells with flattened nuclei which were in a monolayer. Six-week-old male and female *CBA/C57BL* mice were killed by stunning and neck fracture. Small (2 mm) pieces of peritoneum were excised and placed on cover slips mounted on agar drops in a Petri dish. Each explant was allowed to become adherent in a thin clot which formed spontaneously. The explants were covered with a medium composed of NCTC-109 and foetal calf serum (4 : 1), and were incubated at 37° C in an atmosphere of 5 per cent carbon dioxide in air. Outgrowth was generally visible on the fourth day, and on the sixth day the tissue fragments were carefully picked off the cover slips, leaving behind a corona of fibroblasts. These were fixed in 95 per cent ethanol, hydrolysed in 1 N hydrochloric acid for 10 min at 60° C, stained in a standardized Feulgen stain for 1 h, dehydrated and mounted in 'XAM' for examination at times 900 under oil immersion using a Wratten *B* green filter.

In order to avoid the problem presented by the multiple chromocentres (Fig. 1) it was decided to search for Barr bodies only in those nuclei which contained either no chromocentres at all, or the chromocentres of which were sufficiently small so as not to be confused with a Barr body. Such nuclei were designated 'clear' (Fig. 2). To be identified as a Barr body a chromatin mass had to be large (about 1μ), compact, well defined, and tangent to the nuclear membrane all along its base. The aspect of it which faced inward on the cell was allowed to vary from

Fig. 1 Fig. 2

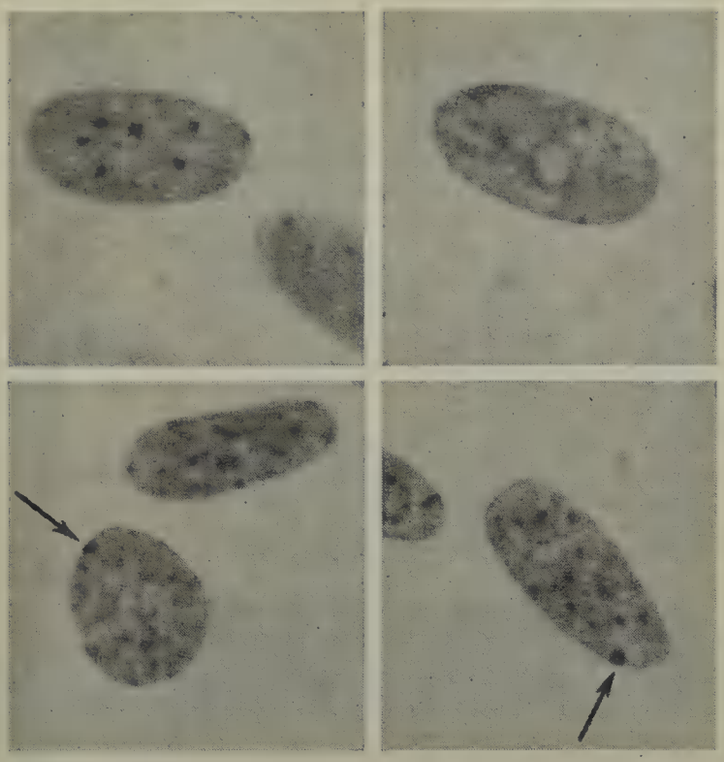

Fig. 3 Fig. 4

Fig. 1. A fibroblast nucleus showing multiple chromocentres
Fig. 2. A 'clear' nucleus with a few very small chromocentres
Figs. 3 and 4. Nuclei containing Barr bodies

a short to a tall pyramid, or to be convex (Figs. 3 and 4). Rectangular masses and masses only slightly tangent to the nuclear membrane were not accepted. All nuclei were examined throughout their volumes.

A preliminary examination of many cover-slip specimens from both male and female animals in accord with these criteria revealed that there was a readily recognizable proportion of female cell nuclei which contained Barr bodies. The corresponding clear nuclei of male cells, on the other hand, did not contain any. These findings were tested further by a review of a series of 'unknown' specimens. Nine mounts were examined independently by two observers who did not know the sex of a given specimen of cells or how many specimens of each sex had been selected for review. Both observers were able readily to identify the sexes of the cell populations. Because subjective factors were numerous it was not felt possible to state exactly the percentage of female nuclei which contained Barr bodies; but after lengthy counts it seemed that about 10 per cent of the clear nuclei contained them. The proportion of nuclei which had to be rejected because of their chromocentre content varied from one preparation to another. In general, somewhat more than half of them were inappropriate for the identification of sex chromatin.

These findings complement the description by Ohno and Hauschka[4] of a heteropycnotic *X* chromosome in late prophase in normal and tumorous mouse tissues, and the recent identifications of a late labelling *X* chromosome in tissue culture of mouse embryo by Galton and Holt[5] and in the living adult mouse by Evans, Ford, Lyon and Gray[6]. It is recognized that the occurrence of Barr bodies in only 10 per cent of fibroblast nuclei is low as compared with the incidence in tissue cultures from other mammals. This seems due in part to the unavoidable loss of some Barr bodies by disregarding all nuclei which bore chromocentres. By the same token it may be related to the low incidence of late-labelling *X* chromosomes reported by Evans and his colleagues, for the recognition of a late-labelling *X* must have been difficult in the presence of the proximal portions of autosomes which labelled equally late. Ohno and Hauschka[4] have suggested that the chromocentres of mouse cell nuclei represent allocyclic portions of many or all the chromosomes of the complement. The variable size of the chromocentres could be attributed to fusion of some of these heterochromatic regions. The 10 per cent of clear nuclei which we have observed to contain Barr bodies may be those of a proportion of cells in which allocycly of one *X* is out of phase with allocycly of the remainder of the chromosomes.

We thank Prof. H. Grüneberg for providing the mice used in the study. One of us (J. D. K.) was supported by the U.S. Public Health Service as an indirect trainee of the National Institutes of Health.

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Electron Microscope Observations of Neurosecretory Elements in the Neurointermediate Lobe in Skates

In this report, observations of ultrastructural organization of neurosecretory elements of the neurointermediate lobe in two types of the Black Sea skates, *Raja clavata* and *Trygon pastinaca*, are considered. As was shown in our earlier work with the light microscope[1], the axoadenar neurosecretory contacts in these types of skates are well developed and very distinct. In the literature there appears to be only one report[2] in which the axoadenar contacts with the adenohypophysis elements in *Scyliorhinus caniculus* (L) were examined with the electron microscope, and some other work done on teleosts[3–5].

With the light microscope, it was found that bundles of the neurosecretory fibres were between the strands of the epithelial glandular cells of the intermediate lobe and elements of connective tissue, and vessels were not found in these spaces. The terminal branches of the neurosecretory fibres are found among the epithelial cells, but never reach the sinusoid capillaries located in the epithelial strands. Numerous swellings of the neurosecretory fibres make intimate contact with the glandular epithelial cells.

During investigations with the electron microscope two kinds of unmyelinated nerve fibres—fibres with elementary neurosecretory granules and those without them—were found in the tissue of the neurointermediate lobe.

The terminal swellings of the neurosecretory fibres—neurosecretory endings—have diameters of considerable size (1·5μ–2μ, more seldom 4·5μ) as compared with the fibres. Neurosecretory granules with various ultra-thin structure and tiny vesicles (diam., 350–500 Å), resembling 'synaptic' vesicles in typical nerve endings, and also mitochondria are found in the terminal swellings in skates as in all other vertebrates, the mitochondria having various but often irregularly curved shapes.

Three main types of neurosecretory granules are observed in the neurosecretory endings (Figs. 1 and 2): (1) Typical elementary granules ranging from 1100 to 2800 Å. (2) Granules of the same size with their central substance having smaller electron density (the degree varying from one granule to another). The distinctness of the light space between the membrane and the central substance is reduced with the osmiophility of the central substance of granules.

(3) 'Residual' neurosecretory granules, as we call them[6], are usually of the same size, but they have membranes which are practically only lipoprotein and are free of the electron-dense central substance. In their structure they resemble vesicles. In some cases, however, we observed the 'residual' granules which are considerably smaller than

elementary granules. Tiny 'residual' granules are almost of the same size as the 'synaptic' vesicles of the neurosecretory endings.

Between all three types of the neurosecretory granules there are connecting transitional forms which, as is considered in the literature, demonstrate the process of dissolving or diluting the substance of a granule during the process of secreting the biologically active substance of the neurosecretory material.

Single neuroglial cells of an elongated form—pituicytes—are found among the neurosecretory fibres (Fig. 2). They are poor in cytoplasm, which surrounds the nucleus only as a thin border (100–300 mμ) and turns into relatively thin branches at its poles. It is in these parts of the cytoplasm that the substantial mass of small mitochondria, thin canals of endoplasmatic reticulum and ribosomes, is concentrated.

The nuclei of pituicytes always have an elongated form—some invaginations often are found on their surface. The nuclei are characterized by a considerable electron density as they contain a large number of chromatin grains. The nucleolus is, as a rule, of a rounded or an oval form and characterized by high electron density.

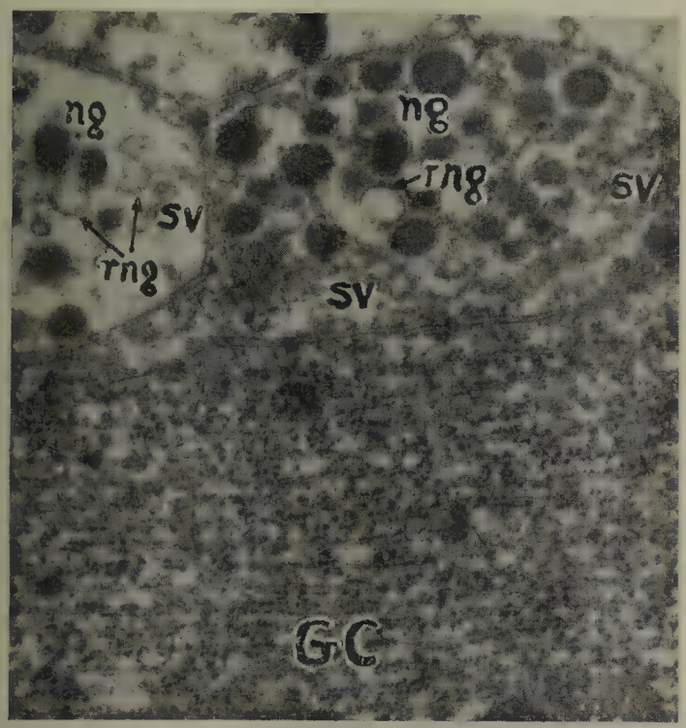

Fig. 1. The contact of two neurosecretory endings with a glandular epithelial cell (*GC*) from the neurointermediate lobe of the hypophysis of *Trygon pastinaca*. Plasma membranes of the terminals and of the glandular cell are divided by a narrow intercellular space about 150 Å. (×37,500)
ng, neurosecretory granules; *rng*, 'residual' neurosecretory granules; *sv*, synaptic vesicles

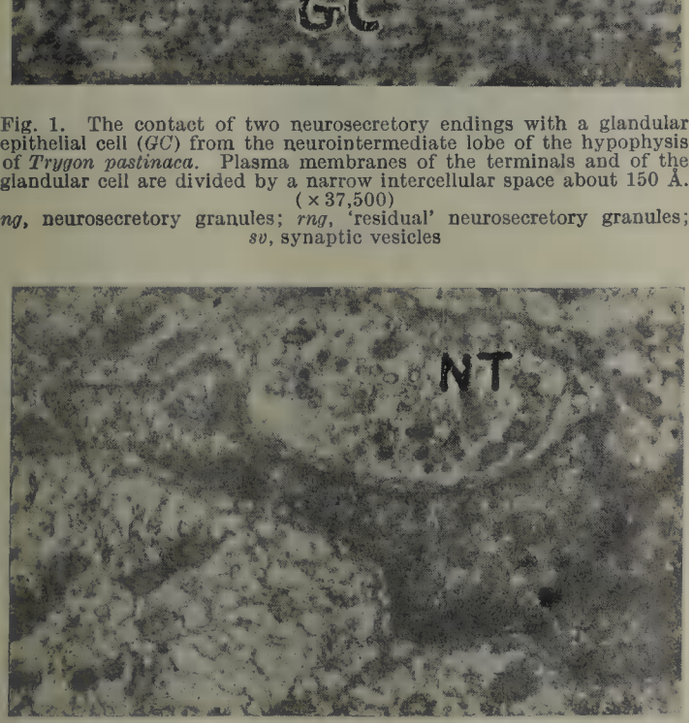

Fig. 2. The contact of the neurosecretory terminal (*NT*) with a pituicyte from a neurointermediate lobe of the hypophysis of *Raja clavata*. (×18,000)

Pituicytes come into close contact with the neurosecretory fibres and sometimes with their terminals. Fig. 2 shows the closest form of such contact where the neurosecretory terminal seems to be invaginated into the body of the pituicytes—the nucleus at the same time being deformed and resembling the invagination form. In their morphology, the pituicytes of skates resemble the oligopituicytes of white mice[7].

The neurosecretory endings are surrounded by glandular cells on all sides. Their plasma membranes are divided only by a thin slit about 150 Å. Some invaginations are found on the surface of glandular cells at the points of contact. No peculiarities have been observed in ultrastructural organization of bounding membrane and organoids of glandular cells in the region of contact with the neurosecretory endings. Compared with typical nerve endings, in the neurosecretory endings there is no concentration of 'synaptic' vesicles near plasmatic membranes which come into direct contact with the surface of a glandular cell. Moreover, no swellings of these sections of membranes similar to those of the presynaptic membrane of typical nerve endings are found.

The foregoing findings concerning the ultrastructure of the neurosecretory endings and the places of contact with the glandular cells testify, we believe, the opinion that the biologically active substances of the neurosecretory granules have a direct influence on the functional activity of the glandular cells of the intermediate lobe. The absence of morphological evidence showing that the neurosecretory granules directly penetrate beyond the endings supports the generally accepted opinion that the biologically active substances of the neurosecretory material—neurohormones—are secreted not as entire granules but as small molecular components.

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Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria: Iguanidae)

The examination of animal chromosomes has recently gained impetus from the development of techniques for the short-term culture of blood[1]. Modifications of this technique have been used successfully on several species of reptiles[2].

Most of the classical literature on reptile chromosomes indicates a conservativeness of karyotype at the familial or super-familial level[3]. Subsequent work by Matthey[4] on the chamaeleontids indicates that in that family the differences in karyotype can be used to help establish species groups.

Using a technique of whole blood tissue culture utilizing only 2–3 drops of blood[5], I have made a preliminary examination of the chromosomes of nine species of *Anolis* lizards in order to assess the value of the karyotype as an additional systematic character.

The genus *Anolis* is taxonomically complex, consisting of approximately 200 species, distributed from South America to Central America and Mexico; and throughout the West Indies and the south-eastern United States. On the basis of differences in the structure of the caudal vertebrae, the genus has recently been divided by Etheridge[6] into two major groups (termed α and β) and

both these groups have been sub-divided into major series of species.

The chromosomes of many reptile species fall into two sharply defined size classes termed macro- and micro-chromosomes[3]. Here only the macrochromosomes will be discussed, for my chromosome spreads of *Anolis* so far give equivocal results for the smaller elements, some of which may be masked by the arms of the larger elements.

The chromosome spreads obtained from blood cultures indicate that *A. carolinensis* (from Louisiana), *A. equestris* (Cuba), *A. luciae* (St. Lucia) and *A. bonairensis* (Bonaire) all have twelve macrochromosomes (Fig. 1). This is in agreement with the published karyotype of *A. carolinensis*[3], the only member of the genus previously examined.

A second group, *Anolis grahami* and *A. lineatopus* (from Jamaica), *A. lineatus* (Curacao), and *A. chrysolepis* (Trinidad), have fourteen macrochromosomes (Fig. 2).

Finally, *A. bimaculatus* (St. Kitts) has still another kind of karyotype in which eighteen to twenty chromosomes appear larger than dots. However, there is no sharp distinction between macro- and micro-chromosomes but instead a graded series from large to small (Fig. 3).

Table 1

Major group	Species	Macrochromosomes
Alpha	**Carolinensis** series	
	carolinensis	12
	equestris	12
Alpha	**Latifrons** series	
	luciae	12
	bonairensis	12
Alpha	**Bimaculatus** series	
	bimaculatus	18–20 (see text)
Beta	**Grahami** series	
	grahami	14
	lineatopus	14
Beta	**Chrysolepis** series	
	lineatus	14
	chrysolepis	14

Macrochromosomal number in 9 species of *Anolis*. Alpha and beta are the major groups of *Anolis*, and the various series are series of species within the groups, all as defined by osteological characters[6].

Table 1 shows that in the four cases in which pairs of species belonging to the same series as defined by Etheridge[6] were examined, there is no variation in macrochromosomal number. The major groups of Etheridge can be distinguished only by the fact that betas have 14 macrochromosomes and alphas do not. Obviously further generalizations must await the examination of additional species within the genus.

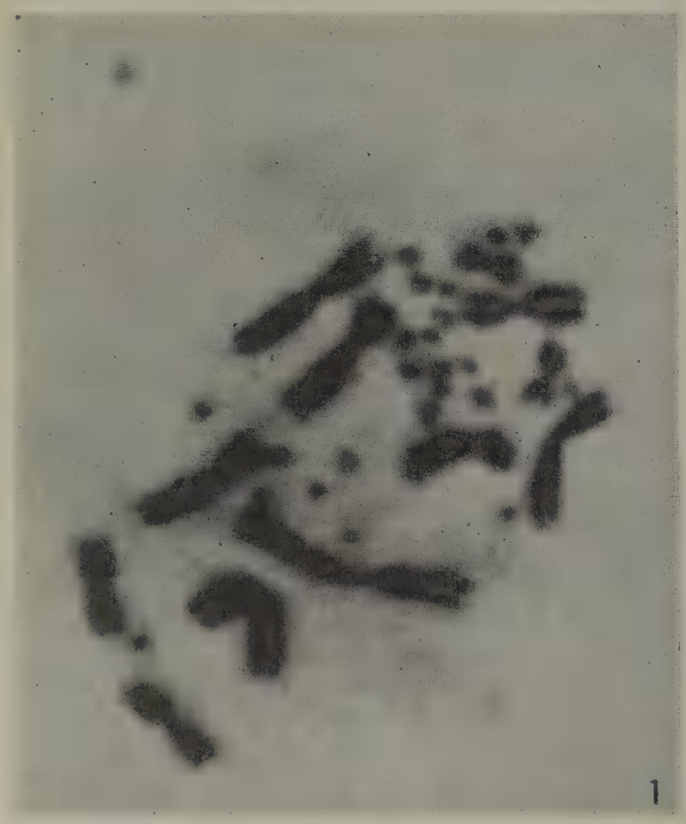

Fig. 1. Chromosomes of *Anolis carolinensis*, showing twelve macrochromosomes

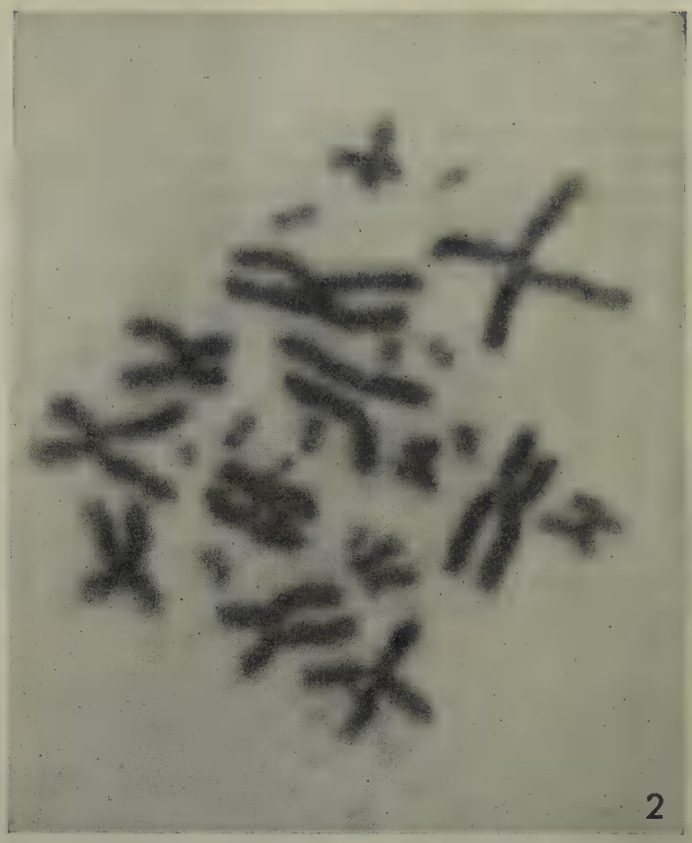

Fig. 2. Chromosomes of *Anolis lineatus*, showing fourteen macrochromosomes

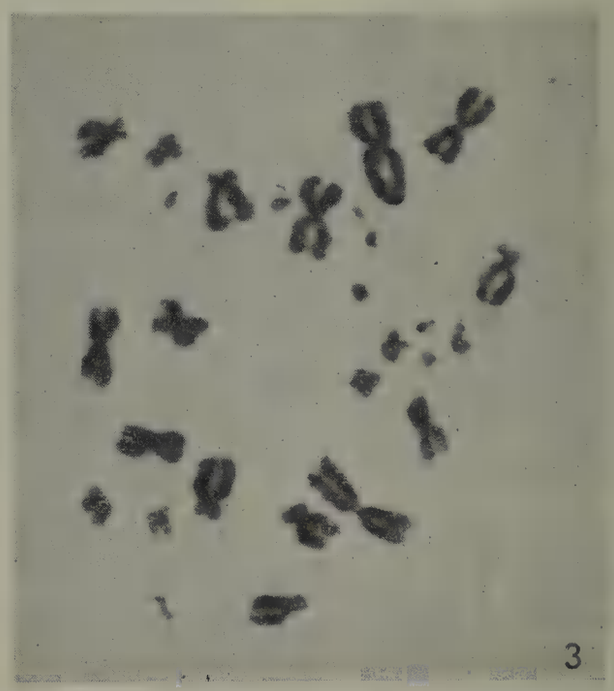

Fig. 3. Chromosomes of *Anolis bimaculatus*, showing a series of chromosomes grading from large to small with no sharp distinction between macro- and micro-chromosomes

These preliminary results suggest that karyotype will be a valuable taxonomic character for the elucidation of relationships and evolution of species groups within the genus *Anolis* as well as in other groups of the Reptilia. The karyotype can be obtained by the relatively simple techniques of blood culture.

I thank Dr. E. E. Williams, Dr. N. Feder and Dr. E. MacLeod of Harvard University for their advice and assistance; and Dr. P. Gerald and Miss S. Warner of the Boston Children's Hospital for introducing me to the techniques of blood culture and for use of their facilities. The photomicrographs were prepared by Dr. Feder. The

work was carried out while I held a pre-doctoral fellowship from the U.S. National Science Foundation.

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GENETICS

Measurements of Bovine Chromosomes

WE recently had an opportunity to study the karyogram of a cell from a bovine male. Since we are interested in sperm nuclear areas in connexion with estimates of DNA in individual sperm nuclei, it was of interest to measure the projected areas of the chromosome images. The comments that follow all derive from the planimetric measurements (three times as complete sets by one observer, without reference to previously recorded measurements) from one karyogram.

The mean total area of the 60 chromosomes (corrected for magnification) was 281·25μ^2, making the mean area per chromosome 4·69μ^2. The smallest chromosome area was 0·203 times that of the largest, 0·400 times that of the mean. The largest area was 1·970 times that of the mean. Bovine chromosomes, except for the presumed sex chromosomes, are so morphologically similar that they cannot always be paired with certainty, so the cumulative distribution of chromosome sizes was plotted on probability (semi-log graph) paper in ascending order of their calculated areas. Except for the seven largest chromosomes, the plot on log-probability paper approximates to a straight line. This is indicative of a skewed distribution of sizes of bovine chromosomes.

The area of the presumed *X* chromosome was 7·85μ^2; in the presumed *Y* it was 3·47μ^2. From these figures, and the total given above, one may compute the expected area of haploid (gametic) sets. A set containing the presumed *X* would have an area of 143·31μ^2 compared with 138·93μ^2 for a set containing the presumed *Y*. Thus there is an expected difference of 3·1 per cent in the area (and probably the mass) of haploid sets of chromosomes bearing either the *X* or *Y*. Could this difference (in *X*- and *Y*-bearing bovine spermatozoa) be detected by measurement of Feulgen-stained chromatin in bovine sperm nuclei? We think not. Using a Barr and Stroud integrating microdensitometer, we obtained a mean standard deviation of 2·033 units for 145 samples of 20 sperm nuclei each. From this one may calculate the expected least significant differences (*L.S.D.*) for different sample sizes. For samples of 1,000 the *L.S.D.* 0·01 turns out to be 0·234 units. For a sample mean of 15 units this is a difference of 1·6 per cent; for a sample mean of 20 units, the difference is 1·2 per cent. Thus it might be possible to detect a difference of 3·1 per cent between two populations of cells provided they were separated. The pooling of two normally distributed populations of equal size, with only a small separation in their means, results in a single population that approximates normality, but may be leptokurtic. To our knowledge there is no means of separating or identifying the *X* and *Y* gametes in bovine sperm populations, despite the attempts of Lindahl[1] by differential centrifugation and Gordon[2] by electrophoresis. The results of Nevo *et al.*[3] would argue against the acceptance of electrophoresis as a general solution to the problem.

One other aspect was considered. Is it within the realm of possibility to see chromosomes in the sperm cell? We think not, despite the claims of Shettles[4] for human material. The measured area in plane of focus of Feulgen-stained bovine sperm nuclei varies from 20 to 35μ^2 (depending on individual variation, treatment of the semen sample, and method of fixation), which is about one-seventh to one-fourth of the area here estimated for haploid sets. Assuming equivalence of mass in the chromosomes of leucocyte cultures and the chromosomes of sperm nuclei, the chromosomes in the sperm nuclei must be layered 4–7 deep, making it unlikely that any optical system could resolve the individual chromosomes. Of course, the calculation above is an over-simplification which does not take into account the range of variability to be expected in the total areas of chromosomes from leucocyte cultures, but we have no reason to believe that the karyogram used was in any way unusual. The work reported was supported in part by a grant, *GM*–08753–03, from the U.S. Public Health Service, National Institutes of Health.

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SOIL SCIENCE

Fixation of Metabolic Products in the Soil during Decomposition of Carbohydrates

SOME of the protein or amino-acids synthesized in the soil exist for a long time because they become resistant towards decomposition. About 40 per cent of the total amount of nitrogen in soils are thus found in amino-acids[1]. This communication describes experiments dealing with the transformation of carbohydrate carbon into amino-acids and other compounds in soils.

Carbon-14-labelled cellulose and glucose were prepared from material originating from barley plants grown to maturity in a growth chamber in an atmosphere enriched with $^{14}CO_2$ (ref. 2). The specific activity of the preparations was about 80 μc./g carbon; the nitrogen content was nil or insignificant. Cellulose (ground to pass a 40 mesh screen) and glucose respectively were added to soil portions in amounts corresponding to 500 mg carbon/100 g of dry soil, besides 10 mg nitrogen in NH_4NO_3 and water to 40 per cent of the water-holding capacity. Samples of 50 g were placed in 100-ml. Erlenmeyer flasks which were stored at 20° C in the dark connected to a respiration apparatus enabling absorption and measurement of the carbon dioxide evolved from the soil. Water evaporated was replenished every second week.

Soil samples were removed with intervals during the decomposition period and analysed as follows. Portions of 50 g without previous drying were shaken for 30 min with 200 ml. of 0·1 N $Ba(OH)_2$ at room temperature. The filtrate of the suspension plus the washings of the residue was neutralized with sulphuric acid. The filtrate from the $BaSO_4$ precipitate was concentrated *in vacuo*, after which the carbon content and the radioactivity of the residue were determined[2]. The soil extracted with $Ba(OH)_2$ was boiled with 6 N HCl for 16 h under reflux, 10 g of air-dry soil plus 100 ml. of acid. The acid extract of the soil combined with the washings of the residue was repeatedly evaporated to dryness *in vacuo*. The part of the residue which was soluble in water was run through a H^+ saturated column of a cation exchange resin ('Amberlite *IR*–120'). The amino-acids retained by the column were desorbed by 2 N ammonia. After removal of the ammonia the concentration of α-amino-acid-N (ref. 3), carbon and radioactivity of the amino-acid solution were determined[2]. Individual amino-acids were

detected on paper chromatograms. Carbon content and radioactivity of the untreated soil and the soil extracted with 6 N HCl were also determined.

Fig. 1 shows the results from an experiment in which cellulose was added to a medium loam soil (*p*H 6·1, carbon 1·4 per cent). It is seen that about 10 per cent of the original cellulose carbon was recovered in amino-acids after 12 and 30 days of incubation; after 90 days of incubation this value had decreased to 7 per cent, and after 300 days to 5 per cent. The amount of original cellulose-carbon incorporated in amino-acids constituted a considerable part of the total amount of original cellulose carbon remaining in the soil, about 40 per cent after 90 days of incubation and 30 per cent after 300 days. Maximum amount of original cellulose carbon in $Ba(OH)_2$ extractable material (2·5 per cent) was observed after 12 days of incubation.

Addition of glucose to the soil mentioned above resulted in a larger incorporation of original glucose carbon in $Ba(OH)_2$ extractable compounds and in amino-acids during the first 5 days of incubation than observed for cellulose, but after 30 days of incubation the amounts incorporated were of the same size as mentioned for cellulose.

The $Ba(OH)_2$ extracted material included a number of free amino-acids and polysaccharides which on hydrolysis yielded glucose and smaller amounts of other sugars. The amino-acids liberated during the treatment with 6 N HCl included 13 normal protein amino-acids besides glucosamine and galactosamine.

The clay content of the soil might be of importance for the fixation of metabolic products[4]. This relation was investigated in a few experiments. A sandy soil (*p*H 5·8, carbon 2·2 per cent) was mixed with washed quartz sand; to the sand was added a clay mineral, Wyoming montmorillonite, American Petroleum Institute Clay Mineral Standard No. 25, obtained from Ward's Natural Science Establishment, Rochester 3, New York. The clay was added in such amounts that the soil–sand mixtures contained 0, 3, 5 and 10 per cent respectively of the clay, besides 50 per cent of the soil, and sand to 100 per cent. Cellulose and glucose were added and the experiments performed and analysed as described above. Before addition the clay was prepared as follows. A 5 per cent suspension of the raw mineral was centrifuged for 10 min at 2,500 r.p.m. The suspension of particles not sedimented was run through columns of Na^+ and H^+ saturated cation exchange resin. The effluent suspension which had a *p*H of 2·2 was titrated to *p*H 5·8 (the *p*H of the soil) with 0·5 N NaOH and centrifuged at 20,000 r.p.m. until all material had come down. The sediment was dried at room temperature *in vacuo* and ground thoroughly.

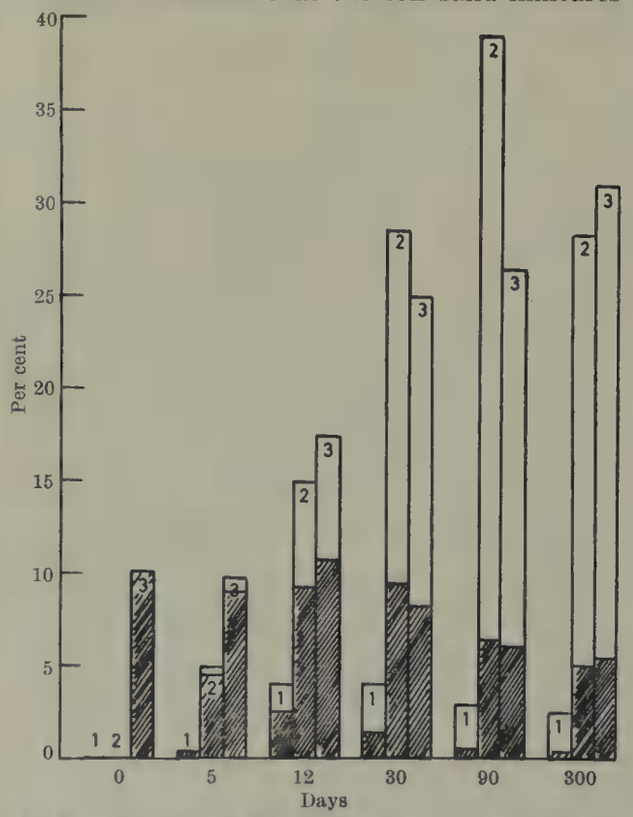

Fig. 1. Transformation of carbon added in cellulose into other compounds during decomposition in soil. The shaded areas of the columns indicate the amounts in per cent of the cellulose carbon added; the total areas of the columns indicate the amounts in per cent of original cellulose carbon remaining in the soil. 'Days' means days of incubation at 20° C. Columns marked 1 indicate original cellulose carbon in material extracted with 0·1 N $Ba(OH)_2$; columns marked 2 indicate original cellulose carbon in amino-acids dissolved during treatment with 6 N HCl; columns marked 3 indicate original cellulose carbon remaining in the soil treated with 6 N HCl

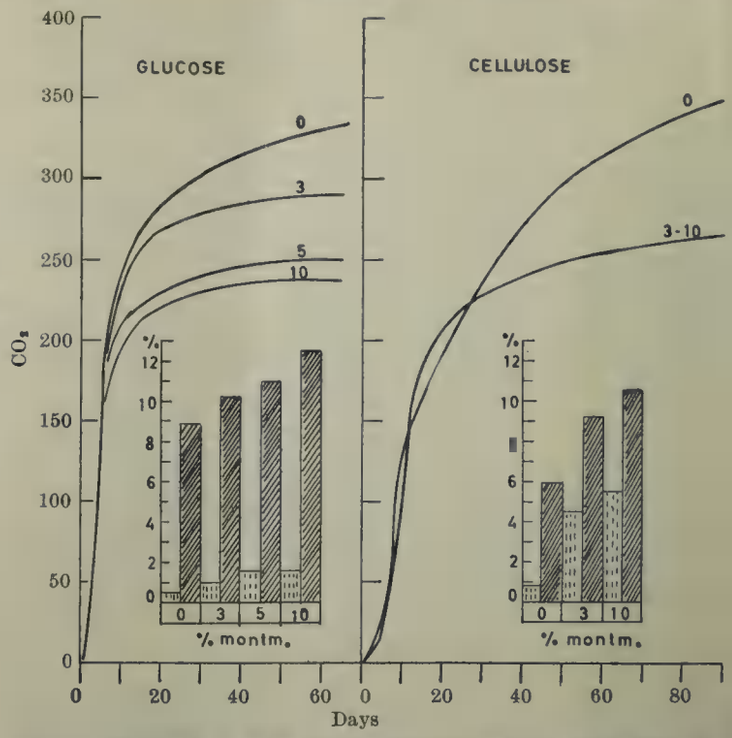

Fig. 2. Influence of added montmorillonite on the decomposition of glucose and cellulose in a soil–sand mixture. CO_2 means mg of carbon in carbon dioxide evolved per 100 g of dry soil–sand, 'days' means days of incubation at 20° C. The curves are summation curves and the numbers indicate per cent of montmorillonite added to the soil–sand from which the carbon dioxide production originates. The inserted histograms show the results of the chemical analyses of the soil–sand performed after 60 (glucose) and 90 (cellulose) days of incubation. The areas of the columns indicate the amounts of original glucose or cellulose carbon recovered in material extracted with 0·1 N $Ba(OH)_2$ (dotted columns), and in amino-acids dissolved during treatment with 6 N HCl (shaded columns). The values are stated in per cent of glucose or cellulose carbon added

Fig. 2 shows that the presence of montmorillonite reduced the rate of the production of carbon dioxide from the soil–sand during the later stage of the decomposition, whereas it seemed to be of no importance during the first stage of decomposition during which about 40 per cent of the added carbon was evolved as carbon dioxide. The rate of carbon dioxide calculated to originate from soil humus was not, or only very slightly, influenced by the addition of the clay mineral.

The chemical analyses of the soil–sand mixtures after the decomposition period (Fig. 2, insets) showed that a larger percentage of the added carbon was recovered in amino-acids where the montmorillonite was added than in the controls without added montmorillonite. This seems to indicate that the reduced rate of production of carbon dioxide was a result of a protection of metabolic products towards microbial decomposition by adsorption to the montmorillonite[5].

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 4

BRITISH BIOPHYSICAL SOCIETY, PHYSICAL BIOCHEMISTRY GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1), from 11 a.m. to 4.30 p.m.—Meeting on "Light Scattering".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "The Teaching of Mechanics of Machines".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (in the Shell Centre Theatre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Film Evening.

Monday, October 4—Wednesday, October 6

IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS (at the Royal Commonwealth Society, Northumberland Avenue, London, W.C.2)—Joint Conference on "Machinability".

Tuesday, October 5

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "Acoustic Excitation and Mechanical Resonance".

Wednesday, October 6

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, London, S.W.7), at 3.15 p.m.—Twenty-ninth Science Meeting. Report on the International Colour Conference in Lucerne (June 1965) and also on the C.I.E. Committee Meeting in Basle. Speakers: Dr. B. H. Crawford, Dr. D. A. Palmer, Dr. R. W. G. Nunt and Mr. F. J. B. Wall.

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Mr. N. Capener: "An English Seventeenth Century Emergency Hospital"; Mr. J. Shepherd: "The Civil Hospitals in the Crimea, 1855–1856"; Mr. D. Ll. Griffiths: "Medicine and Surgery in the American Civil War".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1) at 5.30 p.m.—Mr. R. Hollinghurst: "Radiation-Resistant Fluids and Lubricants".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Filling Gaps in V.H.F./U.H.F. Service Areas" opened by Mr. L. F. Tagholm.

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—"Photomicrography—a Brains Trust".

ENVIRONMENTAL GROUP (in the Department of Electrical Engineering, Imperial College of Science and Technology, Exhibition Road, London, S.W.7), at 5.45 p.m.—Extraordinary General Meeting. 6.30 p.m.—Discussion Meeting on "Existing Methods of Appraisal".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. H. W. Cole: " 'SECAR'—a Modern SSR Ground Interrogator and Decoding Equipment".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Mr. L. G. Goodwin: "Comparative Pharmacology".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Dr. J. Pendered: "Shock Response Analysis Under Shock Spectrum".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Prof. T. S. West: "Some Special, Sensitive and Selective Reactions in Inorganic Trace Analysis".

TELEVISION SOCIETY (at the I.T.A. Conference Suite, 70 Brompton Road, London, S.W.3), at 7 p.m.—Discussion Meeting on "The Technical Future of British Television".

Thursday, October 7

ROYAL SOCIETY OF MEDICINE, UNITED SERVICES SECTION (at 1 Wimpole Street, London, W.1), at 5 p.m.—Surgeon Commander J. D. Walters and Dr. J. J. Brand: "Motion Sickness".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Dr. G. W. Scott Blair: "The Subjective Assessment of the Consistency of Materials in Relation to Physical Measurements".

Friday, October 8

BIOCHEMICAL SOCIETY (at the Middlesex Hospital Medical School, London, W.1)—453rd Meeting. Programme includes a Colloquium on "The Effects of Hormones on Nucleic Acid Synthesis".

Saturday, October 9

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. J. Eric S. Thompson: "The Ancient Maya Civilization of Central America".*

Monday, October 11

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (Joint Conference with the Schools Council, at 1 Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m.—Conference on "Applied Science and Engineering Activities in Schools".

UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Drews (University of Freiburg): "The Cell of the Photosynthetic Bacteria in Light- and Dark-Metabolism" (further lecture on October 13).*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Germany: a Regional Geography" (colour film).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL BIOCHEMIST (with a good honours degree in chemistry or biochemistry and/or a Ph.D. or other higher qualification) in the Department of Chemical Pathology, Ashton Street, Pembroke Place, Liverpool, 3—The Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool, 1 (October 12).

PHYSICIST, BASIC GRADE, IN THE PHYSICS DEPARTMENT, to assist with the routine, development and research work of the department in the application of physics to medical problems (if necessary training will be given in all aspects of this work)—The House Governor, The London Hospital, Whitechapel, London, E.1 (October 14).

ASSISTANT LIBRARIAN (qualified librarian preferably with some experience in a college library)—The Clerk to the Governors, Woolwich Polytechnic, Wellington Street, Woolwich, London, S.E.18 (October 15).

PROGRAMMING ASSISTANT IN THE COMPUTING DEPARTMENT, to write programmes for the University KDF 9 computer and to assist in the general supervisory service of the Department—The Secretary of the University Court, The University of Glasgow, Glasgow (October 15).

RESEARCH ASSISTANT IN THE DEPARTMENT OF GEOGRAPHY to undertake the field work and photogrammetric plotting of aerial photographs necessary to compile a series of maps of an area in the Inner Hebrides—The Secretary of the University Court, The University of Glasgow, Glasgow, W.2 (October 15).

LECTURER (initiated, designed, constructed and used electronic devices, not necessarily medical) IN BIOMEDICAL ENGINEERING—The Secretary, The University, Aberdeen (October 16).

LECTURER (with special qualifications in social geography or social anthropology) IN GEOGRAPHY—The Registrar (Room 39, O.R.B.), The University, Reading (October 16).

SENIOR LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN STATISTICS; a LECTURER IN COMPUTATION; and a LECTURER or ASSISTANT LECTURER IN OPERATIONAL RESEARCH—The Registrar, Ref. 124Y/E, Bradford Institute of Technology, Bradford, 7, Yorkshire (October 18).

ASSISTANT STATISTICIAN (with an honours degree in statistics, or a first- or second-class honours degree (or equivalent qualification) in which statistics is a principal subject) in the Department's Headquarters in London—B. Johnston, Department of Education and Science, Curzon Street, London, W.1 (October 19).

LECTURER or ASSISTANT LECTURER (preferably with research experience, interest or qualifications in platyhelminth or nematode parasitology or the ecology and systematics of small animals) IN THE DEPARTMENT OF ZOOLOGY, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 20).

RESEARCH ASSISTANT (with an honours degree in botany or an equivalent qualification, with an interest in taxonomy and preferably with some knowledge of bryophytes) IN THE DEPARTMENT OF BOTANY for participation in a project involving floristic and phytogeographical studies in antarctic bryophytes—The Assistant Registrar (Science), The University, Birmingham 15 (October 22).

LECTURER (with special qualifications in economic geography and quantitative methods) IN THE DEPARTMENT OF GEOGRAPHY, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

SENIOR LECTURERS and LECTURERS IN THE DEPARTMENT OF PHYSICS, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

LECTURER IN GEOLOGY AT FOURAH BAY COLLEGE, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 28).

READER IN COMPUTER SCIENCE AT THE INSTITUTE OF COMPUTER SCIENCE, to lead a small research group concerned with the general design and use of future computing systems including problems of man-machine communication—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 29).

SCIENTIFIC INFORMATION OFFICER (with a botanical or agricultural degree, the ability to write correct English, and preferably a knowledge of foreign languages) AT THE COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS, Cambridge, for work which includes abstracting, editing, classification of biological literature and dealing with scientific enquiries—The Director, Commonwealth Agricultural Bureaux, School of Agriculture, Cambridge (October 30).

JUNIOR LECTURER or LECTURER (with at least a B.Sc. (Hons.) degree, and preferably some experience in radioisotope work) IN SOIL SCIENCE, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

LECTURER or SENIOR LECTURER (with a degree in agricultural science or science, and experience in soil fertility work, either in the field or the laboratory) IN SOIL SCIENCE at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

SENIOR LECTURER, LECTURER or JUNIOR LECTURER (one or two appointments) (preferably with research experience or special interests in (*a*) plant physiology, particularly of the cell and tissue levels, (*b*) morphology and physiology of lower plants; or (*c*) cytology and histochemistry) IN THE DEPARTMENT OF BOTANY, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

CHAIR OF HAEMATOLOGY at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 1).

CHAIR OF PLANT PHYSIOLOGY within the Waite Agricultural Research Institute, University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, November 1).

LECTURER (graduate in microbiology or biochemistry) IN BACTERIOLOGY—The Secretary, Trinity College, Dublin, 2, Republic of Ireland (November 1).

LECTURER (with a higher degree in anthropology/sociology, experience in conducting independent research, and preferably some teaching experience) IN ANTHROPOLOGY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 13).

SENIOR LECTURER (competent circuit engineer with modern experience and a knowledge of digital computers, and preferably analytical ability and lecturing experience) IN ELECTRICAL ENGINEERING at the University of

Adelaide, South Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, November 15).

Assistant Lecturers or Lecturers (3) in the Department of Physics—The Registrar, The University, Liverpool, quoting Ref. CV/290 (November 27).

Lecturers (with a good honours degree and a keen research interest in any branch of electronics and control engineering) in the School of Engineering Science, Electronics and Control Division—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

Assistant Lecturer, Grade B, to teach human anatomy, physiology and hygiene and possibly some general science, together with biology to G.C.E. Advanced Level—The Registrar, Derby and District College of Technology, Kedleston Road, Derby.

Assistant Lecturer (honours graduate with suitable postgraduate experience) in Physics—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

Assistant Lecturer (with appropriate qualifications in biology, mycology or botany, and preferably special qualifications in plant mycology or general mycology) in Biology in the Department of Applied Microbiology and Biology—The Registrar, University of Strathclyde, Glasgow, C.1.

Chief Technician (with appropriate technical qualifications and wide practical experience) in the School of Biological Sciences at Bath, to be responsible for the overall control of technical services and the ordering of materials and equipment in the School—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST. 65/90.

Electronics Technician (preferably with a National Certificate in Electrical Engineering or equivalent) in the Biological Laboratory, to be responsible for the maintenance and construction of electronic instruments used in research and teaching—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

Field Assistant (Female) (with a degree in zoology, preferably with an interest in geography or geology) at Preston Montford Field Centre, near Shrewsbury—The Secretary, Field Studies Council, 9 Devereux Court, Strand, London, W.C.2.

Graduate Research Officer (preferably with experience in medical statistics, biometry or genetics) in Statistics in the Oxford Record Linkage Study and Unit of Clinical Epidemiology—The Medical Director, Oxford Record Linkage Study, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford.

Lecturer (honours graduate in zoology with subsidiary qualifications or interest in physiology) in Zoology in the Biology and Zoology Department—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27.

Lecturer or Assistant Lecturer (preferably with a special interest in social statistics, social administration or industrial sociology) in Sociology, to assist with teaching for London honours degrees in Sociology—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

Librarian (qualified person or graduate)—The Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford.

Master (preferably graduate in mechanical sciences or engineering) to teach Mathematics and some Physics—The Headmaster, Oundle School, Peterborough.

Master to teach Mathematics to scholarship level—The Headmaster, Monkton Combe School, near Bath, Somerset.

Ornithologist (university graduate in zoology) with the Canadian Wildlife Service, Quebec City, to undertake ecological studies of migratory bird populations with particular emphasis on the distribution, numbers, locations and migrant patterns of waterfowl and the evaluation of their habitat—Technical and Scientific Requirements Group, Civil Service Commission of Canada, Ottawa 4, Ontario, Canada, quoting Ref. 65–1350K.

Physicist (well qualified and experienced, able to teach to university scholarship level)—The High Master, Manchester Grammar School, Manchester, 13.

Physicist (with an honours degree) in the Physics Department for work involving the clinical and biological uses of ionizing radiation—The Physics Department, Westminster Medical School, Horseferry Road, London, S.W.1.

Professor in Physical Chemistry—Prof. Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

Research Assistant (civil or chemical engineering graduate) in the Department of Civil Engineering, to investigate fluidization-flocculation processes in water purification—The Secretary, University College, Gower Street, London, W.C.1.

Research Assistant in the Department of Physics for research on plasma generation by high power lasers—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex.

Research Assistant (with a good honours degree in sociology) in Sociology—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

Soil Chemist (national of the United Kingdom or the Republic of Ireland, with either an honours degree in agricultural chemistry (soils and plants), or a degree in chemistry with postgraduate experience in agricultural analysis (soils and plants), and preferably experience in soil and plant analysis) with the East African Common Services Organization, to carry out and supervise soil and plant analysis and to help develop and investigate new methods—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/04.

Technician or Senior Technician (with a good knowledge of general laboratory practice, and preferably special experience in experimental and microscopical cell studies or electron microscopy) in the Analytical Cytology Section, to assist in work on cell ultrastructure and function—A. D. Greenwood, Department of Botany, Imperial College, London, S.W.7.

Technician (preferably with experience in the food or pharmaceutical industries) to take Charge of Biochemical Engineering Pilot Plant—The Establishment Officer, University College, Gower Street, London, W.C.1, quoting Ref. Chem. Eng./3.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Agricultural Research Council. A Bibliography of Farm Buildings Research. Part 1: Buildings for Pigs, 2nd Supplement, 1962–64. Pp. 80. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 5*s*. 6*d*. net. [268

Political and Economic Planning. Annual Report 1964–65. Pp. 12. (London: Political and Economic Planning, 1965.) [268

Automatic Computing: Its Problems and Prizes. By Prof. Stanley Gill. (Inaugural Lecture, 26 January, 1965.) Pp. 69–93+6 plates. (London: Imperial College of Science and Technology, 1965.) 4*s*. 6*d*. [268

Building Research Station Digest (Second Series). No. 61: Strength of Brickwork, Blockwork and Concrete Walls. Pp. 6. (London: H.M. Stationery Office, 1965.) 4*d*. [268

Agricultural Research Council: Soil Survey Research Board. Soil Survey of Great Britain—Report No. 17, 1964. Pp. v+46. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 6*s*. net. [268

Wye College (University of London). Annual Report of the Department of Hop Research 1964 (from 1st April, 1964, to 31st March, 1965). Pp. v+99. (Wye, Ashford, Kent: Wye College, 1965.) 6*s*. [268

Ministry of Technology: Laboratory of the Government Chemist. Report of the Government Chemist 1964. Pp. viii+108+4 plates. (London: H.M. Stationery Office, 1965.) 8*s*. net. [268

Geological Survey and Museum. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Huntingdon and Biggleswade (Explanation of One-inch Geological Sheets 187 and 204, New Series). By E. A. Edmonds and the late C. H. Dinham, with contributions by J. B. W. Day and Dr. R. Casey. Pp. viii+90+3 plates. (London: H.M. Stationery Office, 1965.) 25*s*. net. [268

The British Drug Houses, Ltd. 2 : 4-Dinitro-phenyl-hydrazine. Pp. 35. (Poole, Dorset: The British Drug Houses, Ltd., 1965.) [268

Other Countries

United States Department of the Interior: Geological Survey Bulletin 1183: The Crystalline Rocks of South Carolina. By William C. Overstreet and Henry Bell, III. Pp. v+126+4 plates. Bulletin 1188: Geology of Northwestern North Park, Colorado. By William J. Hail, Jr. Pp. v+133+plates 1–5. Bulletin 1199-B: Bauxite and Kaolin Deposits of Mississippi, Exclusive of the Tippah–Benton District. By Louis C. Conant. Pp. v+70+plates 1–5. (Washington, D.C.: Government Printing Office, 1965.) [268

Proceedings of the United States National Museum, Smithsonian Institution. Vol. 117, No. 3508: Microlepidoptera of Juan Fernandez Islands. By J. F. Gates Clarke. Pp. 1–106. Vol. 117, No. 3509: Neotropical Hemerobiidae in the United States National Museum. By W. Nakahara. Pp. 107–122. Vol. 117, No. 3510: Hermatobates, a New Generic Record for the Atlantic Ocean, with Descriptions of New Species (Hemiptera: Gerridae). By Jon L. Herring. Pp. 123–130. (Washington, D.C.: Government Printing Office, 1965.) [268

Western Australia. Report of the Government Chemical Laboratories for the year 1963. (Extract from the Report of the Department of Mines.) Pp. 39. (Perth: Government Printer, 1965.) [268

Contributi dell'Istituto Geofisico e Geodetico dell'Università di Genova all' Anno Geofisico Internazionale 1957–58 e 1959–1963. Memoria Nr. 3: Ricerche sulla Radiazione Solare. Da M. Bossolasco, G. Cicconi, I. Dagnino, A. Elena e G. Flocchini. Pp. 56. (Genova: Istituto Geofisico e Geodetico dell'Università di Genova, 1965.) [268

Progress in Protozoology: Abstracts of Papers read at the Second International Conference on Protozoology, London, 29th July–5th August, 1965. (International Congress Series, No. 91.) Pp. 278. (Amsterdam, New York, London, Milan, Tokyo and Buenos Aires: Excerpta Medica Foundation, 1965.) 65*s*. [268

Proceedings of the United States–Japan Conference on Research Related to Earthquake Prediction Problems. (Sponsored by the National Science Foundation and Japan Society for Promotion of Science.) Pp. 105. (Tokyo: Dr. T. Hagiwara, Earthquake Research Institute; New York: Dr. J. Oliver, Lamont Geological Observatory, Palisades, 1964.) [268

Year Book of the National Institute of Sciences of India, 1965. Pp. vi+239. (New Dehli: National Institute of Sciences of India, 1965.) Rs. 15. [268

United States Department of the Interior: Geological Survey. Professional Paper 490: Geology of the Flaming Gorge Area, Utah–Colorado–Wyoming. By Wallace R. Hansen. Pp. viii+196+plates 1–3. (Washington, D.C.: Government Printing Office, 1965.) [268

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 46 (June 1965): Education and the Occupation of Men. Geographic Pattern of Fatal Accidents. Further Reduction in Maternal Mortality. Hospital Care of Company Personnel in 1964. Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.) [268

Cenco Instruments Corporation. 1965 Annual Report. Pp. 34. (Chicago: Cenco Instruments Corporation, 1965.) [268

Atlantide Report No. 8: Scientific Results of the Danish Expedition to the Coasts of Tropical West Africa, 1945–1946. By Jørgen Knudsen and Torben Wolff. Pp. 255. (Copenhagen: Danish Science Press; London: British Museum (Natural History), 1965.) 65 D.kr.; 65*s*. [268

University of Lagos. Change in Vice-Chancellorship. Pp. 28. (Lagos: The University, 1965.) [268

Records of the Queen Victoria Museum, Launceston. New Series, No. 18: Thomas Bock's Portraits of the Tasmanian Aborigines. Pp. 24 (10 plates). New Series, No. 19: Two Skink Lizards Newly Recorded from Tasmania. By R. H. Green. Pp. 4. (Launceston: The Queen Victoria Museum, 1965.) [268

Records of the Australian Museum. Vol. 26, No. 6 (5th June, 1964): The Archaeology of the Capertee Valley, New South Wales. By Frederick D. McCarthy. Pp. 197–246+plates 11–24. 16*s*. 6*d*. Vol. 26, No. 14 (6th January, 1965): Some Nephtyidae (Polychaeta) from Australian Waters. By Kristian Fauchald. Pp. 333–340. 2*s*. (Sydney: The Australian Museum, 1964 and 1965.) [268

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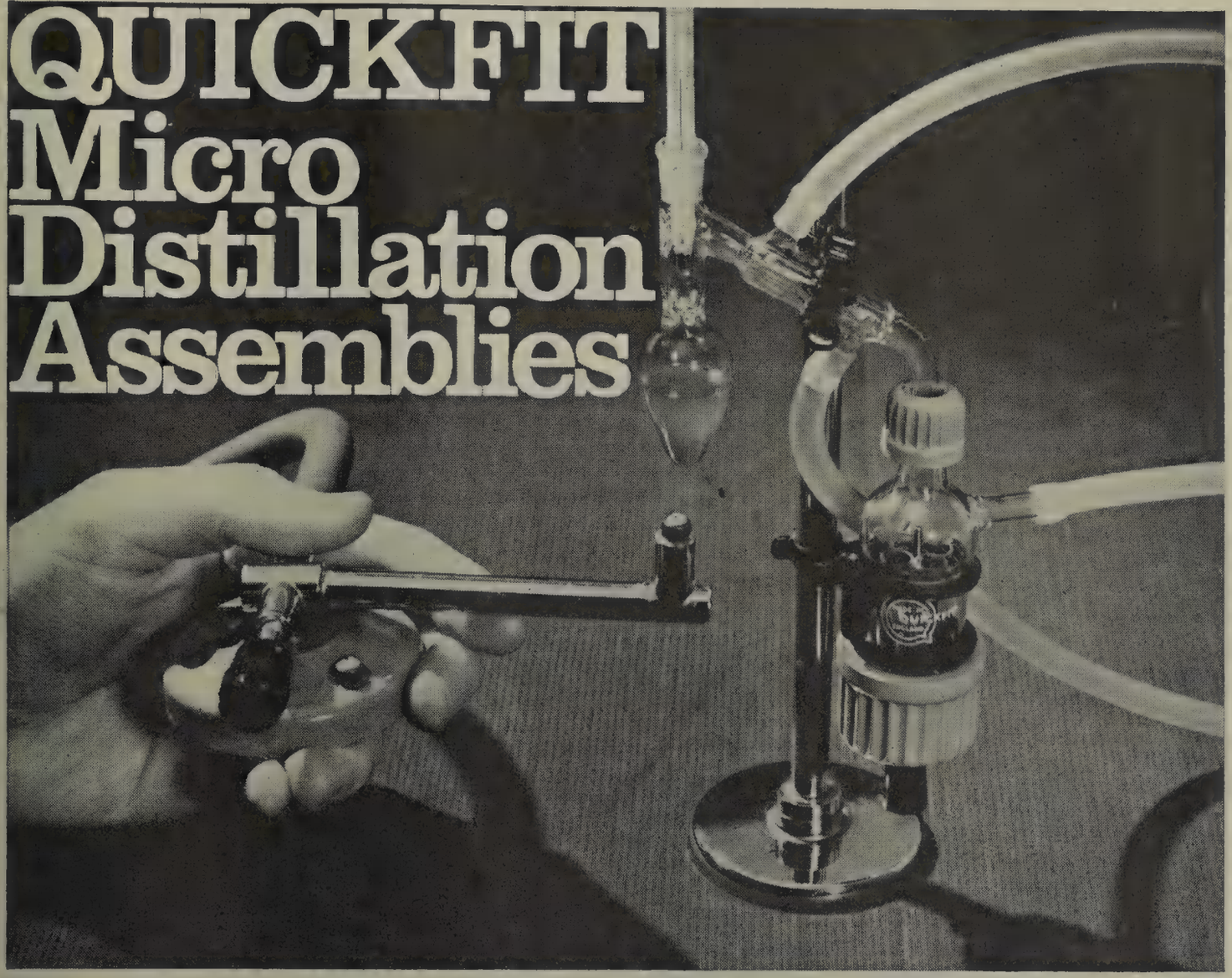

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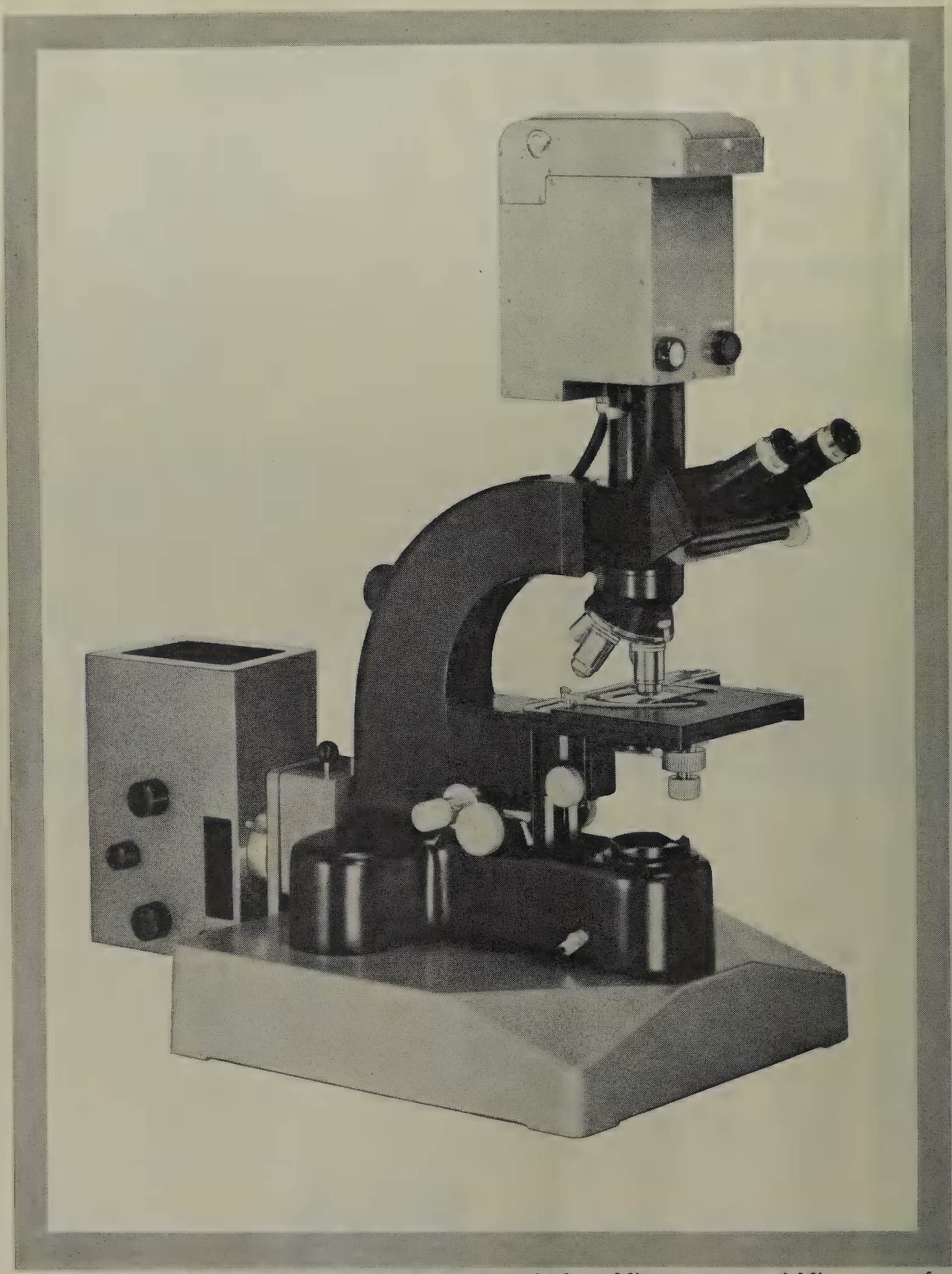

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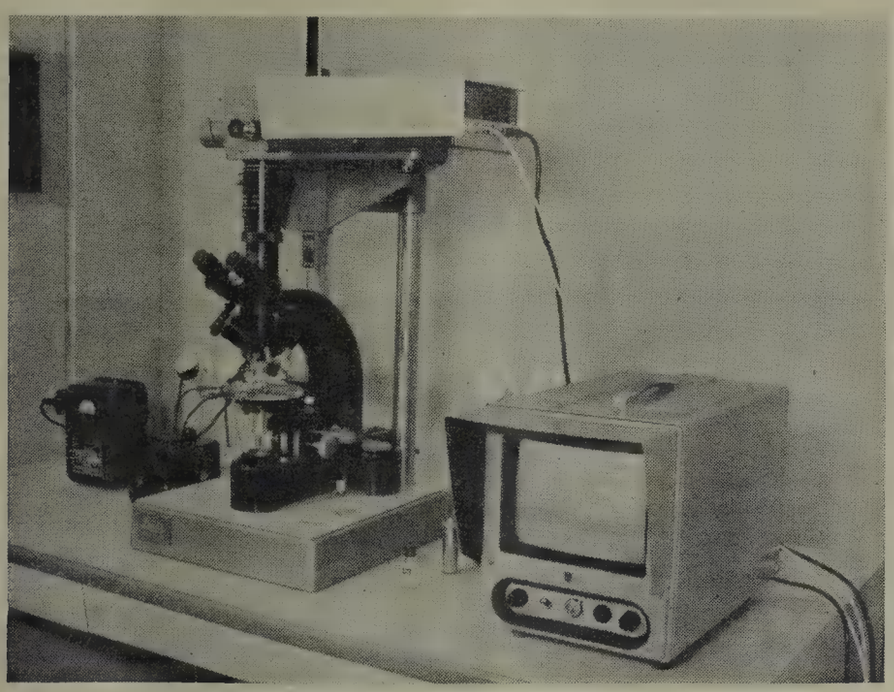

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Incidental expenses: Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the New Zealand Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, the Secretary, State Services Commission, P.O. Box 8004, Wellington, New Zealand, with a closing date of November 15, 1965.

NEW ZEALAND DEPARTMENT OF EDUCATION

CENTRAL INSTITUTE OF TECHNOLOGY

SCHOOL OF PHARMACY

Applications are invited for the position of Head of the New Zealand School of Pharmacy as from January, 1966, or as soon thereafter as possible. Applicants should be suitably qualified and experienced to supervise teaching and ancillary staff and 150 students following a full-time pharmacy course.

The present salary is £2,390 per annum. A rental house is available and the successful applicant may join the Government Superannuation Scheme.

Payment of fares and expenses for the appointee and family will be made, subject to certain details as set out in "Information for Applicants" which will be supplied on request.

Further details of the position and of the conditions of service may be obtained from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, or the Director of Education, Private Bag, Wellington, New Zealand.

UNITED BIRMINGHAM HOSPITALS

THE CHILDREN'S HOSPITAL
LADYWOOD ROAD, BIRMINGHAM, 16

SENIOR GRADE AND BASIC GRADE BIOCHEMISTS

HOSPITAL AND RESEARCH APPOINTMENTS

Required for Biochemistry Department. Experience in Paediatric Biochemistry not essential. The person appointed will be given a general training and encouraged to specialize in some aspect of the subject. One appointment will be exclusively for research into pyridoxine and Tryptophan metabolism. Opportunity for a higher degree if required. Salary, Basic Grade, £830 to £1,175; Senior Grade, £1,780 to £2,210.

Apply, giving the names of two referees, to Dr. D. N. Raine.

AUSTRALIAN ATOMIC ENERGY COMMISSION

RESEARCH ESTABLISHMENT,
LUCAS HEIGHTS,
SYDNEY, AUSTRALIA

RESEARCH SCIENTIST

Applications are invited from chemists interested in radiochemistry, analytical chemistry, and physico-chemical instrumentation for the vacancy of Officer-in-Charge of the Low Level Radiochemistry Group. This group is engaged in the measurement of very low levels of radioactive nuclides in a wide range of materials including waters, biological samples, soils, etc. The work involves a programme of research and development of new methods of radiochemical analysis.

Excellent facilities are available. Publication of research work is encouraged.

Applicants should possess a degree in chemistry at the Ph.D. level or qualifications and scientific research experience which taken together are equivalent thereto; they should have demonstrated a capacity for independent research and have a breadth of scientific interest which extends beyond their original specialised training.

The commencing salary depends upon qualifications and experience and will be within the following ranges:

Research Scientist £A2,445 to £A3,105, or
Senior Research Scientist £A3,235 to £A3,755.

Application forms and further information may be obtained from the Atomic Energy Adviser, A.A.E.C. Office, Australia House, Strand, LONDON, W.C.2.

Early application is desirable, preferably before October 31, 1965.

rhm

YOUNG SCIENTISTS

Ranks Hovis McDougall Limited wish to appoint a number of young scientists in the Applied Research Section at their Central Cereal Laboratory, London.

The appointments cover a wide range of research work, including the development of tests for quality control procedures in wheat and flour. There are good opportunities for promotion within the Group's diverse and increasing scientific interests.

Candidates should be graduates or should have other appropriate qualifications in chemistry, biochemistry or physics. Experience in cereal laboratory work would be an advantage, but those without previous experience will be equally considered.

Good starting salaries will be offered, and there is a generous pension scheme.

Applications, quoting reference No. 46/65, with details of age and experience to :—

The Staff Manager (Recruitment)
Ranks Hovis McDougall Ltd
Millocrat House, 53, Eastcheap, London, E.C.3.

HUNTINGDON RESEARCH CENTRE

Due to the continued rapid expansion of this research organization there are now further vacancies for male and female RESEARCH ASSISTANTS and ANIMAL TECHNICIANS in all grades for interesting and varied work in the general field of drug evaluation. Previous experience with animals is an advantage, but this is not essential as full training will be given and facilities for further study are available.

Good salaries will be paid to the right applicants and benefits include three weeks holiday annually.

Applications, which will be treated in confidence, should be made in writing to the Administrative Officer, Huntingdon Research Centre, Huntingdon, quoting reference H/N/11/T.

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF ENGINEERING

It is hoped shortly to appoint an experienced Research Engineer in the Control Group of the Engineering Department. This post, which carries the status of an Assistant Director of Research, is associated with the expanding programme, on Government Contract, for the application of control theory to industrial process optimization. A knowledge of control theory and its application to Engineering problems is required, preferably added to practical engineering experience. Preference will be given to applicants with higher degrees. Salary scale £1,740 by £85 to £2,505.

Further details and application forms from the Secretary of the Faculty Board of Engineering, Trumpington Street, Cambridge.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF PROTEIN CHEMISTRY

POST-DOCTORAL FELLOWSHIP IN ELECTRON MICROSCOPY

Applications are invited from Chemists, Biologists or Physicist with experience in electron microscopy for appointment to a Post-doctoral Fellowship in Electron Microscopy tenable with the Organization's Division of Protein Chemistry, located at Parkville, Melbourne, Victoria.

The Division has a research staff of some 30 organic and physical chemists, biochemists and biophysicists, engaged in studies of the structure and chemistry of fibrous proteins and their soluble derivatives and precursors. The equipment available for these studies includes a Siemens electron microscope, a Hilger microfocus X-ray diffraction unit, a Beckman IR9 infra-red spectrometer, a Varian e.s.r. spectrometer and in addition a wide range of physico-chemical and analytical techniques are available.

Applicants should have a Ph.D. degree or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

SALARY: Dependent upon qualifications and experience within the ranges of: £A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

The Fellowship will be initially for a period of three years. Consideration will be given to the continuation of F.S.S.U. arrangements.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of the appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No: 462/236) should be addressed by November 20 1965.

Glaxo Laboratories Ltd. Greenford.

Entomologist

To meet the Company's expanding interest in parasitology applications are invited from Entomologists with post-graduate research experience in the field of entomology. The work will be concerned with the testing of insecticides and will be carried out at the Greenford Laboratories and at the Research Farm at Harefield, Middlesex. It is not envisaged that anyone under 30 years of age would have the necessary experience and a post-graduate qualification would be an advantage.

The Company offers attractive conditions of employment, a generous salary, pension scheme and the opportunity to share in the Company's profitability. Applications should be made to: The Personnel Manager, Glaxo Laboratories Ltd., Greenford, Middlesex.

PEDOLOGIST

required by

AN FORAS TALUNTAIS (The Agricultural Institute) for the National Soil Survey Department of its Soils Division.

Appointment up to Senior Research Officer level. Marriage and children's allowances. Superannuation Scheme, including special arrangement for F.S.S.U. member.

ESSENTIAL: Honours University Degree or equivalent in Agriculture, Horticulture, Forestry, Geology, or Geography.

APPLICATIONS CONSIDERED FROM 1965 FINAL DEGREE CANDIDATES.

Further particulars and application forms from Director, An Foras Taluntais, 33 Merrion Road, Dublin 4. Latest date for receipt of completed forms—October 13, 1965.

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF MATHEMATICS

CHAIR OF NUMERICAL ANALYSIS

Applications are invited for the newly created Chair of Numerical Analysis. The salary for the post will be within the professorial range with F.S.S.U. benefits.

Details of the appointment may be obtained from the Registrar, Glasgow, C.1, to whom applications should be lodged by October 25, 1965.

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF MATHEMATICS

Applications are invited for:

LECTURESHIPS IN NUMERICAL ANALYSIS

These posts are associated with the creation of the Chair of Numerical Analysis advertised above.

LECTURESHIPS IN STATISTICS

A LECTURESHIP and an ASSISTANT LECTURESHIP IN PURE MATHEMATICS

Candidates should have a research interest in some branch of their subject. The salary range for a Lecturer is £1,400 by £85 to £2,505 and for an Assistant Lecturer £1,050 by £75 to £1,275 with F.S.S.U. benefits. The initial salaries of the persons appointed will depend upon qualifications and experience.

Application forms and further particulars can be obtained from the Registrar, Glasgow, C.1, with whom applications should be lodged by October 25, 1965.

LABORATORY ASSISTANT

(ASSAY LAB.)

A vacancy will shortly occur in our Microbiological Laboratory. Candidates should have "A" Level G.C.E. in Chemistry and Mathematics. Previous experience of Microbiological Assay work useful but not essential — training will be given. Laboratories situated near Tadworth, Surrey, and transport available from Tadworth station.

Apply Assistant Personnel Manager, Vitamins Ltd., 23 Upper Mall, London W.6, quoting reference LA/WOs.

HEINZ 57 SCIENTISTS FOR RESEARCH AND DEVELOPMENT

Heinz need scientific staff for research into food processing and packaging at their new research centre recently opened in pleasant parkland at Hayes, Middlesex. Laboratory and pilot shop facilities are excellent. However, research and investigations cannot be carried out with modern equipment alone. They need more well-qualified scientific staff, specifically:

BIOCHEMISTS · CHEMICAL ENGINEERS CHEMISTS · FOOD SCIENTISTS PHYSICISTS · PHYSICAL CHEMISTS MICROBIOLOGISTS

Work covers most technological aspects of food processing and, in particular, the Physicist/Physical Chemist will be engaged on investigations into technological aspects of food packaging. Applicants should have at least a good honours degree or appropriate Diploma of Technology. Research experience or Ph. D. would be an asset for the Biochemical post. Ideally the upper age limit in all categories is 32, although this is by no means inflexible. Salaries and working conditions are those of a large progressive company. Financial assistance in changing location can be granted in certain cases.

Apply in writing, giving a brief personal history, to:
The Personnel Officer,
H. J. HEINZ COMPANY LTD.
Hayes Park, Hayes, Middlesex

FISHERIES RESEARCH BOARD OF CANADA

RESEARCH SCIENTISTS IN STATISTICAL ECOLOGY

Applications are invited for positions as Senior Research Scientists (biology, statistics, mathematics) in research laboratory being developed in the Halifax area of Nova Scotia to study mechanisms underlying natural marine production. Main programme will be to outline the distribution, abundance, population dynamics and trophic relations of major populations of pelagic and benthic fish species and to discover the biological basis for the observed relationship. Demonstrated ability for independent research and previous supervisory experience essential. Salary will be up to $12,000 (Canadian) per year, depending on qualifications, with higher salaries in exceptional cases. Usual Canadian Government Superannuation and other benefits available.

Further inquiries should be sent to: The Director, Fisheries Research Board of Canada, Atlantic Oceanographic Group, Bedford Institute of Oceanography, Box 1006, Dartmouth, N.S., Canada.

SOUTH AUSTRALIA

BOTANIC GARDENS

Applications are invited for appointment to the position of Botanist with the above-named Government department. Salary, up to £A2,252 per annum (male) or £A2,054 (female) depending on qualifications and experience. Qualifications: Applicants should hold a University Degree, preferably majoring in Botany. Applications will be considered from applicants with extensive knowledge of taxonomy and herbarium experience. Duties: General herbarium duties with subsequent specialization and research in several plant groups, determination of plants, supervision of botanical assistants in herbarium, and other duties as directed by the Keeper of the Herbarium. Passages to South Australia arranged. Housing available on rental basis. Conditions include recreation, sick and long service leave. Superannuation Fund available.

For further details and application forms apply to: The Agent General and Trade Commissioner for South Australia, South Australia House, 50 Strand, London, W.C.2. WHItehall 7471.

PA Management Consultants Ltd.
PERSONNEL SERVICES DIVISION

DIRECTOR OF RESEARCH

GEIGY (U.K.) LIMITED

Geigy's contribution to dyestuffs, pigments, pharmaceuticals and agricultural and industrial chemicals spans over 200 years; recognition of its research work has included the award of a Nobel Prize. The present research effort is centred on Switzerland, U.S.A. and the U.K. The review of research policy has led to a decision to intensify the research effort within Geigy U.K., which at present covers industrial chemicals, pigments and pharmaceuticals.. The new post of Director entails Board level responsibility and calls for an Organic Chemist with high qualifications, who has already indicated that he has outstanding ability in original research in fields of interest to Geigy. Such a man will enjoy the stimulus of working within the world-wide structure of Geigy, free to frame his own programme, but able to draw on the body of knowledge and experience already available. The salary, other benefits and prospects will also prove attractive to him.

(Ref. AA5/1777/N.)

The identity of candidates will not be revealed to our client without prior permission. Applicants should forward brief details, quoting the reference number, to :—

P. A. Management Consultants, Ltd.,
Personnel Services Division,
Knightsbridge, London, S.W.1.

UNIVERSITY OF QUEENSLAND

The University invites applications for the following positions. The salary ranges are:

Senior Lecturer	£A3,200 by £A100 (6) to £A3,800
Lecturer	£A2,400 by £A100 (7) to £A3,100
Senior Demonstrator	£A1,900 by £A50 (5) to £A2,150
Demonstrator	£A1,500 by £A50 (4) to £A1,700

SENIOR LECTURER IN PHYSICS, AND THEORETICAL PHYSICS

A Senior Lecturer should have a Ph.D. in physics (or equivalent in research publications) and some university teaching experience. He should have an intimate knowledge of some branch of physics, to the extent that he is competent to lecture on it to graduates and Final Honours undergraduates. One position is reserved for applicants having experience in theoretical physics.

SENIOR LECTURER IN HIGHWAY ENGINEERING

Applicants should be Honours graduates in civil engineering and have had some professional engineering experience. Applicants shall have undertaken postgraduate study in highway and/or traffic engineering. Experience in the design of highway structures is desirable.

SENIOR LECTURER/LECTURER IN ANTHROPOLOGY

Applicants should have an appropriate degree and should have completed or nearly completed a higher degree. Candidates with interests and research in the sociology of complex or simple societies will be considered.

LECTURER OR SENIOR LECTURER IN GEOPHYSICS

An applicant should have a science degree specialising in geophysics or related discipline and some research experience. He should have recent experience in exploration geophysics. The appointee will be expected to teach and develop modern exploration geophysics and to have an interest in some other aspects of solid earth geophysics. There will be a share of the Departmental field training and such other duties as the Head of the Department may require. He will be encouraged to do his own research and will be expected to assist in the supervision of postgraduate students in geophysics.

LECTURER IN MICROBIOLOGY

Applicants should hold a Master's (or higher) degree in science, agriculture, dentistry, or medicine, Majoring in bacteriology or plant virology, and have had postgraduate experience in research. Teaching experience would be an advantage.

SENIOR DEMONSTRATOR/DEMONSTRATOR IN ANTHROPOLOGY

Applicants should hold a degree in anthropology or sociology, preferably with Honours.

The University provides for permanent staff, superannuation similar to F.S.S.U., housing assistance, study leave, and travel grants.

Additional information and application forms will be supplied upon request to the Association of Commonwealth Universities, Marlborough House, Pall Mall, London, S.W.1. Applications close in London and Brisbane on October 29, 1965.

WYETH

Patent Department

Applications are invited from Organic Chemists or Biochemists with a good Honours degree, for a vacancy in the patents department which has arisen because of the working expansion of one of the leading ethical pharmaceutical manufacturers.

This is an excellent opportunity for a scientist to enter the profession of patent agency under conditions where his scientific qualification will be fully recognized and where he will receive training to enable him to qualify as Chartered Patents Agent.

Candidates should preferably be not more than 28 years of age. Previous patent experience whilst not essential, would be an advantage. Conditions of employment are excellent and the Company operates a non-contributory Group Life Assurance and Pension Fund.

Please send full details of age, experience and present position together with an indication of availability for interview to the Personnel Manager, John Wyeth and Brother Limited, Huntercombe Lane South, Taplow, Maidenhead, Berks.

SCIENTIST

PREFERABLY A PHYSICIST OR PHYSICAL CHEMIST

is required for a fundamental research group carrying out various studies of a basic, or even academic, nature on a material of everyday use.

Structural studies involve the application of light electron and X-ray microscopy as well as other more specialized techniques appropriate to the immediate field of study. Physical properties of interest cover, for example, rheology, light scattering and surface smoothness.

The person appointed should be of graduate status with some research experience, not necessarily in the paper industry. It is essential that he be capable of applying basic thinking to relate results of fundamental studies to aspects of an applied nature. He should also be prepared to extend basic knowledge into practice in order to advance the understanding of paper manufacture, structure, behaviour and usage.

Applications to: The Director (ref.: PAT), The British Paper and Board Industry Research Association, St. Winifred's Laboratories, Welcomes Road, Kenley, Surrey.

ENTOMOLOGIST

BRITISH HONDURAS

Required to advise the Agricultural Extension Staff and farmers on pest control measures, to evaluate insecticides and undertake basic field research in pest control, to lecture on pests and pest control and to advise on legislation to control the use of certain insecticides, and pest control generally.

Candidates must possess a B.Sc. degree with Honours in Zoology or Entomology. Practical experience of control of field pests and pests of stored products is desirable.

Salary in the range £1,170 to £2,047 a year. 12½ per cent gratuity. Two- to three-year contract. Passages provided. Government quarters. Education allowances.

Candidates should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/26/03, to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Assistant Lecturer in Genetics in the Departments of Botany and Zoology in the Faculty of Science. Candidates should have a wide interest in the subject so as to be able to lecture to students of both departments. Duties to commence as soon as may be arranged. Salary on a scale £1,050 to £1,275 per annum, with membership of the F.S.S.U. Initial salary according to qualifications and experience.

Applications should be sent not later than October 20, 1965, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained on quoting reference 174/65/Na.

IMPERIAL COLLEGE

NUCLEAR MAGNETIC RESONANCE STUDIES IN ORGANIC CHEMISTRY

A vacancy exists for an Assistant Lecturer or Lecturer, depending on qualifications and experience, in Organic Chemistry. The research interests of the candidate should be in nuclear magnetic resonance spectroscopy and both 60 and 100 megacycle instruments will be available.

Applications, with curriculum vitae and the names of two referees, should be sent to Professor D. H. R. Barton, F.R.S., Department of Chemistry, Imperial College, London, S.W.7.

STC RECTIFIER DIVISION

Senior Engineer
FILM DEPOSITION
£1,300-£1,700

This vacancy results from the introduction of novel methods for film deposition and would ideally be filled by an engineer with a knowledge of Chemistry and Physics. Duties will include process and equipment development evaluation of film properties and areas of application of the process and equipment, and will involve liaison with our Research organization. Age range is not important but applicants should be qualified to at least H.N.C. and have a minimum of 5 years development engineering experience.

Graduate Engineer
FILM DEPOSITION
£975-£1,350

This is an ideal opportunity for a young engineer holding a degree in Chemistry or Physics, with little or no post-graduate experience, to join the above section, where the variety and challenge of the techniques involved make this a sound foundation for successful career development.

Graduate Engineers
INFRA-RED FILTERS
£1,100-£1,350

These are career opportunities for young graduates with perhaps two years development experience to enter the interesting field of research and development of visible and infra-red filters. The work will involve computation of filter characteristics, investigation of new materials and techniques for their deposition, development of processes and equipment to achieve improved characteristics and the evaluation of the properties of developed filters.

The above vacancies are created by sustained expansion of this Company, and consequently offer a sound and progressive future; starting salaries will be within the ranges indicated. The division is pleasantly situated on the Herts–Essex border, but within easy reach of London; we offer assistance with removal expenses and in obtaining mortgage facilities, and rented housing is available in the new town. All applications will be treated in strict confidence, and preliminary interviews, without commitment, may be arranged during the evening or week-end if required; write initially to

L. J. PLUMBER, PERSONNEL MANAGER,
STC RECTIFIER DIVISION, EDINBURGH WAY, HARLOW, ESSEX

microanalyst

Required in the Organic Chemistry Division of the National Chemical Research Laboratory, C.S.I.R.

SOUTH AFRICA

The successful applicant wil be required to take charge of the routine microanalytical operations of the laboratory.
Experience in microelementary and functional group analysis is essential and familiarity with the operation and maintenance of electronic equipment will be a recommendation.
Salary: In accordance with qualifications and experience between R.2,520 (£1,260) and R.3,120 (£1,560) per annum.
Written applications, reflecting age, marital status, qualifications and experience should be sent to:
The South African Scientific Liaison Officer, Chichester House, 278 High Holborn, London, W.C.I.

Electron Microscopy and Diffraction Techniques

Applications are invited from Senior Scientists experienced in the application of Electron Microscopy and Electron Diffraction Techniques to the study of the structure of solids.

Electron microscopy is being increasingly used to further our research on the role of both lattice defects in crystals and micro structure in general, as well as on the physics and chemical properties of materials in bulk and thin film form.

Experience of dislocation physics, the strength of solids and diffraction theory would be an advantage.

This is a senior appointment within the Research Department of the company. A realistic salary will be paid and applications from scientists living abroad are welcome.

Apply, in confidence,
to the Staff Manager,
MORGANITE RESEARCH AND DEVELOPMENT LIMITED,
Battersea Church Road,
London, S.W.11.

Mullard

METALLURGIST

We have a vacancy in the Mullard Central Materials Laboratory for a Metallurgist of graduate or equivalent level to join a team working on materials used in the electronics industry. This work involves the preparation and fabrication on a laboratory scale of a wide range of new or uncommon metals or alloys; the study of properties of metals and the investigation of new techniques involved in the application of metals. Although previous experience in a similar industrial laboratory is desirable, applications from people who may not have this experience but do have a good basic knowledge of metallurgy will be considered.

Applicants are, therefore, invited to write, with a brief summary of their career to date, to:—

The Personnel Officer,
THE MULLARD RADIO VALVE COMPANY,
MULLARD LIMITED,
New Road, Mitcham Junction, Surrey.

quoting reference CML/KBL.

LIVERPOOL EDUCATION COMMITTEE

CITY OF LIVERPOOL COLLEGE OF TECHNOLOGY
BYROM STREET, LIVERPOOL 3
Principal: S. A. J. Parsons, B.Sc.(Econ.), M.I.Mech.E., M.I.Prod.E., M.B.I.M.
DEPARTMENT OF CHEMISTRY AND BIOLOGY

Applications are invited from well-qualified candidates for the following posts, duties to commence as soon as possible.

F/62S: TWO SENIOR LECTURERS IN INORGANIC CHEMISTRY.—Candidates must be prepared to teach inorganic chemistry to Honours B.Sc. level, and to initiate and direct research; for one post, experience in X-ray crystallography would be an advantage.

F/63S: LECTURER IN PHYSICAL CHEMISTRY.—To teach chemistry to Honours B.Sc. level.

F/64S: LECTURER IN BIOCHEMISTRY.—To teach biochemistry to Honours B.Sc. level.

For all posts candidates must be interested in carrying out research, and teaching or industrial experience are desirable.

Salaries: Senior Lecturer, £1,895 to £2,115 per annum (under review); Lecturer, £1,670 to £1,895 per annum (under review).

Application forms and further particulars can be obtained from the Director of Education, 14 Sir Thomas Street, Liverpool, 1, on receipt of an addressed foolscap envelope. Return forms to the Principal of the College not later than October 11, 1965.

THOMAS ALKER,
Town Clerk.

UNIVERSITY OF MANCHESTER

The Department of Metallurgy requires an Experimental Officer to undertake work in electronics. Much of the research work of the Department utilizes electronic equipment. Whilst some of this can be purchased, much of the equipment must be designed and constructed for a particular function. This requires a man who is knowledgeable about electronic techniques and devices and is able to apply this knowledge to the designing of circuits which must then be constructed, tested, and put into operation. The Experimental Officer will also be expected to maintain electronic equipment within the Department and to advise on all future purchases of such apparatus. Applicants should preferably possess H.N.C., Dip.Tech., or some professional qualification, including electronics. Experience is essential. The initial salary will be within the range £870 to £1,450 per annum, according to qualifications and experience.

Applications should be submitted by October 23, 1965, to the Registrar, the University, Oxford Road, Manchester 13, from whom further particulars and forms of application may be obtained on quoting reference 175/65/Na.

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE

Applications are invited for two posts of Lecturer in the Department of Anatomy. Candidates need not be medically qualified, although such a qualification would be an advantage. Applications from recent graduates who wish to take up an academic career will be welcomed. The successful candidates will be expected to take part in the teaching of medical and science students. The department possesses extensive facilities for research particularly in the fields of reproductive physiology, neurology, endocrinology, comparative anatomy and radiobiology. Ample time will be available for the persons appointed to undertake research, either under the direction of senior workers in the department, or in fields of their own choosing. Salary scales: £1,400 by £120 to £2,000: £2,000 by £120 to £2,600 for Lecturers with medical degrees; £1,400 by £85 to £2,165; £2,165 by £85 to £2,505 for Lecturers without medical degrees, in each case with F.S.S.U. participation.

Applications (nine copies), naming two referees, should be sent to the Assistant Registrar, Medical School, Birmingham 15, by October 31, 1965. Persons interested in these posts are invited to visit the Department of Anatomy. Arrangements may be made by writing to the Professor of Anatomy (Sir Solly Zuckerman, K.C.B., F.R.S.).

WESTMINSTER MEDICAL SCHOOL

Physicist required in Physics Department. Work involves clinical and biological uses of ionising radiation. Opportunities for research available. Honours degree in physics essential. Appointment on the N.H.S. basic grade (£980 to £1,665).

Further details from the Chief Physicist. Applications, stating age, qualifications, experience, and names of two referees, to Physics Department, Westminster Medical School, Horseferry Road, London, S.W.1.

COMMONWEALTH AGRICULTURAL Bureaux. Applications are invited for the post of Scientific Information Officer at the Commonwealth Bureau of Plant Breeding and Genetics, Cambridge. Work includes abstracting, editing, and classification of biological literature and dealing with scientific inquiries. Applicants should possess a botanical or agricultural degree, the ability to write correct English, and preferably knowledge of foreign languages. Salary scale £895 to £2,349. Starting salary according to age, qualifications and experience. Two extra increments after two years' satisfactory service for applicants aged 32 or under at date of appointment. Provision for superannuation.—Applications, stating age, qualifications, present salary and other particulars, and names of referees (work and character) should be submitted by October 30, 1965, to the Director of the Bureau, School of Agriculture, Cambridge, from whom further particulars are obtainable.

UNIVERSITY OF DUBLIN
TRINITY COLLEGE
DEPARTMENT OF PHARMACOLOGY

RESEARCH LECTURER OR SENIOR RESEARCH LECTURER IN MEDICAL STATISTICS: The appointee will be responsible for the statistical design and evaluation of the investigations in Clinical Pharmacology carried out in the Department of Pharmacology and associated hospitals. He will be encouraged to carry out his own research in medical and social statistics. He will be responsible for arranging a course in statistics for medical students in their premedical and clinical curricula, and will be available for giving statistical advice to other Departments in the School of Physic. He will be the representative of the School of Physic in the Department of Mathematics. Salary scale: Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,100; Research Senior Lecturer, £1,850 by £60 to £2,450 per annum.

JUNIOR RESEARCH LECTURER OR RESEARCH LECTURER IN CLINICAL PHARMACOLOGY: This post will be combined with an appointment as Honorary Registrar in the Federated Dublin Hospitals, and will initially be made for a three-year period. Membership of one of the Royal Colleges of Physicians and/or postgraduate experience in clinical research is necessary. Duties will include teaching and research on the clinical applications of drugs and the design and arrangement of therapeutic trials. Salary scale: Junior Research Lecturer, £1,000 by £50 to £1,200 per annum; Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,150 per annum.

JUNIOR RESEARCH LECTURER OR RESEARCH LECTURER IN CLINICAL PSYCHOPHARMACOLOGY: Applications are invited for these posts which will be established in the near future. The post which is filled will be combined with an appointment as Honorary Registrar in St. Patrick's Hospital, Dublin, and will initially be made for a three-year period. A Diploma in Psychological Medicine and/or postgraduate experience in clinical psychiatric research is necessary. Duties will include teaching and research on the clinical applications of drugs and the design and arrangement of therapeutic trials, with special reference to the use of drugs in psychiatric medicine. Salary scale: Junior Research Lecturer, £1,000 by £50 to £1,200 per annum; Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,150.

RESEARCH ASSOCIATE IN CLINICAL PHARMACOLOGY: This post is open for application by students who wish to register for a postgraduate degree in order to carry out research on the clinical applications of drugs in general or psychiatric medicine in hospitals associated with the Department of Pharmacology. Candidates should be medical students of the University of Dublin who have passed their B.A. examination, or should have an Irish Medical degree. Basic salary scale £450 to £500 per annum.

RESEARCH ASSOCIATE IN CHEMICAL PHARMACOLOGY: This post is open for application by candidates who wish to register for the degree of M.Sc. or Ph.D. in order to carry out research on chemical aspects of drug metabolism. Candidates should have an honours degree in organic chemistry or biochemistry. Salary scale £450 to £500 per annum.

Candidates possessing medical qualifications will receive an annual increment of £100 on their basic salary.

For all these appointments (excepting those of Research Associate) starting salaries may be varied according to qualifications and experience. Superannuation contributions are paid by the College, together with marriage and child allowances of £65 per annum and £45 per annum. Further particulars from the Secretary to the College, Trinity College, Dublin, 2. Closing date for applications, October 30, 1965.

SENIOR TECHNICIAN REQUIRED IN THE Department of Chemical Pathology to assist in the investigation of sick children. Salary scale, £805 to £1,677, with placing according to qualifications and experience.—Applications, with names of two referees to Chemical Pathologist, Hospital for Sick Children, Institute of Child Health, Great Ormond Street, London, W.C.1. The Chemical Pathologist will be pleased to answer any queries by letter or telephone, Holborn 9200.

UNIVERSITY OF ABERDEEN. SENIOR Technician or Technician required for Department of Biological Chemistry, Marischal College. National Certificate, City and Guilds or other appropriate qualification required. Salary in range £840 to £1,040 and £600 to £840 per annum.—Apply in writing to the Secretary, Marischal College, from whom further particulars may be obtained, by October 9, 1965, quoting Reference No. 166/65.

NEW ZEALAND

Applications are invited for the undermentioned vacancy:

DIRECTOR, TAIERI AGRICULTURAL RESEARCH CENTRE: DEPARTMENT OF AGRICULTURE, NEW ZEALAND

The Taieri Agricultural Research Centre is situated in the South Island, approximately 1½ miles from Mosgiel and 8 miles from the city of Dunedin and the University of Otago. The Centre consists of (a) the Invermay Research Station, (b) the Taieri Soil Research Station and (c) The Taieri Veterinary Diagnostic Station, which together have a total staff of approximately 50 including 11 scientific officers.

An area of approximately 1,300 acres is available for experimental purposes. Of this, 200 acres is first-class alluvial soil, deep and fertile, highly suited for agronomic studies; much of the remainder is undulating clay down country eminently suited for work with sheep and beef cattle.

Duties: To control and direct the existing activities of the centre and to develop an expanded programme of soil, plant and animal research with particular reference to the problems of the South Island.

Qualifications desired: A high degree of scientific leadership and the ability to plan and supervise an expansion of the research centre's research programme and facilities. Evidence of a distinguished research record in either plant, soil or animal science must be produced. Previous administrative experience desirable, but not essential.

Salary: In the range of £2,700 to £3,100 according to qualifications and experience with the possibility of further promotion which will be dependent on attainment of the Department's development objectives and the demonstration of a high standard of scientific ability and leadership.

Conditions of appointment: The standard conditions of assistance to New Zealand apply, i.e., surface fares to Taieri, New Zealand for self and family; assistance towards accommodation expenses for a limited period in New Zealand until the successful applicant finds housing and up to £100 for a married man and £35 for a single man for removal expenses for personal effects. Payment of fares and expenses is subject to bond to serve in the New Zealand Public Service for a period of three years.

Further details and application forms may be obtained from the Director, Agricultural Research, Ruakura Agricultural Research Centre, Private Bag, Hamilton, New Zealand, quoting reference 3901.

Applications will close with the Secretary, State Services Commission, P.O. Box 8004, Wellington, New Zealand by November 15, 1965.

UNIVERSITY OF MELBOURNE

LECTURER IN THE DEPARTMENT OF ZOOLOGY

Applications are invited for the above-mentioned position. Preference will be given to applicants with experience in comparative and cellular physiology. Duties will include lecturing to second- and third-year students in these fields and conducting laboratory classes. The department has excellent modern facilities for electron-microscopy, electrophysiology, histo- and cyto-chemistry. The head of the department is Professor G. Burnstock, Ph.D. (Professor M. J. D. White, D.Sc., F.A.A., F.R.S., is Head of the School of Zoology and Genetics.) It is desirable that the appointee should be able to take up the position as soon as possible after January 1, 1966. Salary: £A2,400 to £A3,150 per annum. Closing date for applications: November 1, 1965. Initial salary will be determined according to qualifications and experience.

Further information, including details of F.S.S.U. type superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

NUFFIELD DEPARTMENT OF CLINICAL MEDICINE

UNIVERSITY OF OXFORD

Applications are invited for the post of Graduate Research Officer in Statistics in the Oxford Record Linkage Study and Unit of Clinical Epidemiology. Experience in medical statistics, biometry or genetics will be advantageous. Computer facilities available. The successful applicant will be encouraged to work for a higher research degree. The post is in the Lecturer and Research Officer Grade; salary £1,400 by £85 to £2,335, point of entry according to age and experience.

Further particulars from the Medical Director, Oxford Record Linkage Study, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF PLANT INDUSTRY

ECONOMIC BOTANIST—PLANT INTRODUCTION

Applications are invited for appointment as Senior Research Scientist or Principal Research Scientist (Economic Botanist) with the Organization's Division of Plant Industry. The appointee will be located at Canberra, A.C.T., Australia.

The Division of Plant Industry has a professional staff of over 170 scientists located at its headquarters in Canberra, A.C.T., and at regional laboratories and field stations in many parts of Australia. Current research embraces many fields of plant biology including agronomy, plant breeding, ecology, microclimatology, plant nutrition, genetics, physiology and biochemistry. Many of the scientists engaged in research in the above fields have close working relationships with the Plant Introduction Section.

The Division has excellent facilities for laboratory and field research including well equipped laboratories, a controlled environment research laboratory, glasshouses, experiment stations, herbarium and library.

The appointee will have two major responsibilities. Firstly he should actively explore means of obtaining, testing and developing plants which are novel to Australian agriculture, or which have not been examined in Australia. Secondly, he should take general responsibility for Plant Introduction, the routine work of which is performed by skilled and experienced staff, involving the collection of plants throughout the world for use by Government and private agencies.

The appointee should have a Ph.D. degree in a relevant field or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability. He should also have a broad knowledge of world flora and a definite interest in agricultural development of an unusual kind. Previous experience in plant introduction is not essential.

SALARY: Dependent upon qualifications and experience within the ranges of:
£A3,235 to £A3,755 per annum or £A3,885 to £A4,485 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

Promotion within C.S.I.R.O. to a higher classification is determined by merit.

The duration of the appointment will be determined in consultation with the successful candidate; some applicants may prefer a fixed-term appointment for three years, in which case consideration will be given to the continuation of F.S.S.U. arrangements.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the conclusion of a fixed-term appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 130/730) should be addressed by October 30, 1965.

INSECT PHYSIOLOGIST OR BIOCHEMIST

"Shell" Research Limited require an Insect Physiologist or Biochemist in their Entomology Division, Woodstock Agricultural Research Centre, Sittingbourne, Kent.

The successful applicant will be expected to conduct research on problems of insect physiology or biochemistry and to investigate the mode of action of insecticides.

Excellent working conditions and technical assistance will be available, with opportunities for publishing original work and for attending scientific meetings. Salary will depend on qualifications and experience.

Applicants should have qualifications of a high order, and preference will be given to applicants with a Ph.D. degree and with experience in the physiology or biochemistry of insects. Applicants at present overseas are invited to apply. The appointment may be taken up immediately, or early in 1966.

Applications in writing giving details of age, qualifications, and experience should be sent to:—

"Shell" Research Limited,
Recruitment Division, (PNDL/11)
Shell Centre, London, S.E.1.

CENTRAL ELECTRICITY GENERATING BOARD

MIDLAND REGION

RESEARCH AND DEVELOPMENT DEPARTMENT

STATISTICIAN

Applications are invited for the post of Statistician in the Midlands Region Research and Development Department located on the Hams Hall Power Station site, Sutton Coldfield, Warwickshire.

The duties will include mathematical work on both the design of and the subsequent analysis of results from a variety of research projects. They involve the use of advanced statistical techniques in the processing of data acquired from research studies and normal power station operation.

The successful applicant will be expected to develop the liaison between the Department and the Regional Computer Branch.

Candidates should have an Honours degree or its equivalent in either mathematics or statistics and should, preferably, have had some experience in a research department. A knowledge of computers will be considered an advantage.

Salary will be in the range £1,550 to £2,385 plus £60 per annum. This maximum rises to £2,470 plus £60 per annum in April, 1966.

Apply in writing, quoting vacancy number 983e/65MR, and giving details of age, qualifications and experience, to the Regional Personnel Officer, 53 Wake Green Road, Moseley, Birmingham, 13, by October 5, 1965.

NATURAL RUBBER PRODUCERS' RESEARCH ASSOCIATION

WELWYN GARDEN CITY, HERTS.

requires an experienced

Electron Microscope Technician

to take charge of the operation and maintenance of its Siemens Elmiskop MKI and ancillary equipment which are housed in a well designed laboratory.

The position is suitable for candidates with experience in biological and physical work and with a sound knowledge of replica, embedding and sectioning techniques. The starting salary will be between £900 and £1,350 per annum, or £1,415 and £1,745 per annum, depending on age, experience and qualifications. Additionally, there are superannuation and children's allowance schemes, and financial help with housing may be granted. The working conditions are congenial. Opportunities for further study are given in suitable cases.

Please write, giving details of qualifications and experience to :

The Director of Research,
N.R.P.R.A.,
56 Tewin Road, Welwyn Garden City, Herts.

SENIOR LABORATORY TECHNICIAN

(Male)

required to join a research group concerned with veterinary studies in the development of new drugs. Preference will be given to holders of an A.I.M.L.T. qualification, or an H.N.C. in Applied Biology.

The post offers interesting work and valuable experience in a well-equipped modern laboratory and would suit a young technician whose main interests are in research.

Applicants should apply in writing, quoting U.159, to the Administration Officer, Wellcome Veterinary Research Station, Frant, Nr. Tunbridge Wells.

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

SCHOOL OF ENGINEERING SCIENCE

ELECTRONICS AND CONTROL DIVISION

Applications are invited for posts of Lecturers in this rapidly expanding School. Candidates with a good honours degree and a keen research interest in any branch of Electronics and Control Engineering will be considered.

Salary will be according to experience and qualifications on the scale £1,400 by £85 to £2,505. In addition there are F.S.S.U. benefits.

Applications (two copies), giving the names and addresses of two referees, should be sent by November 30, 1965, to the Registrar, University College of North Wales, Bangor, from whom further particulars may be obtained.

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY

ASSISTANT LECTURESHIP IN BIOLOGY

Applications are invited from candidates with appropriate qualifications in Biology, Mycology or Botany. Salary on scale £1,050 to £1,275 plus F.S.S.U. Candidates with special qualifications in Plant Mycology or general Mycology will be preferred.

Application forms and further particulars may be obtained from the Registrar, Glasgow, C.1.

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for Lectureship in Department of Geography. Special qualifications in Economic Geography and Quantitative Methods required. Appointment to commence as soon as possible. Salary scale: £1,200 to £2,175 per annum. Family passages, F.S.S.U., children's and car allowances, part-furnished accommodation, regular U.K. leave.

Detailed applications (six copies), naming three referees by October 25, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

UNITED LIVERPOOL HOSPITALS

DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited for the post of Principal Biochemist in the Department of Chemical Pathology at Ashton Street, Pembroke Place, Liverpool 3. The salary scale is £2,255 a year, rising by annual increments to £2,900 a year. Whitley Council conditions of service apply and the post is superannuable. Candidates should possess a good honours degree in Chemistry or Biochemistry and/or Ph.D. or other higher qualification. The post carries considerable responsibility in a well-equipped department which undertakes fundamental research and specialized metabolic and endocrine investigations in the associated metabolic unit.

Applications, stating age, qualifications, experience and the names of three persons to whom reference may be made to the Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool 1, by October 12, 1965.

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MEDICAL PHYSICS

Applications are invited for the post of Lecturer in Biomedical Engineering (Electronic). Applicants must have initiated, designed, constructed and used electronic devices (not necessarily medical). Salary on scale £1,400 to £2,505 with placing according to qualifications and experience. Superannuation (F.S.S.U.) and removal allowance.

Further particulars should be obtained from the Secretary, the University, Aberdeen, with whom applications (ten copies), should be lodged by October 16, 1965.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited for the post of Senior Laboratory Administrator in the Department of Anatomy. Salary scale £1,120 to £1,460. Duties will be those of an administrative assistant to the Professor of Anatomy and will include the regulation of technical services and the routine maintenance of laboratory stores and accounts. Typing services available. Previous administrative experience essential and laboratory or medical knowledge desirable. The appointee will be required to become a member of the Federated Superannuation Scheme for Universities.

Applications, in writing, giving age, education, other experience and the names of three referees, to be sent in by October 23, 1965, to the Registrar, University College, Cathays Park, from whom further particulars may be obtained.

WEST OF SCOTLAND AGRICULTURAL COLLEGE

Applications are invited for a post of LECTURER IN BACTERIOLOGY from candidates with a degree in Bacteriology or equivalent qualification. The salary will be (according to age, qualifications and experience) on one or other of the following scales: Grade III, £1,349 rising to £2,119; Grade IV, £869 (honours £895) rising to £1,234.

Conditions of appointment and application forms are obtainable from the Secretary, The West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2, with whom applications should be lodged not later than October 15, 1965.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Experimental Officer in the Department of Audiology and Education of the Deaf. Candidates should be young graduates interested in the design and construction of electronic and electro-acoustic equipment. Salary range: £870 to £1,450 per annum. The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 30, 1965, to the Registrar, the University, Manchester 13, from whom further particulars may be obtained, on quoting reference 179/65/Na.

CHARING CROSS HOSPITAL AND MEDICAL SCHOOL

Applications are invited for the post of Experimental Officer to work on electronic instrumentation in the field of Cardiology. At a later date the scope of the work will widen to include other medical applications of electronics. Further details of the work may be obtained by writing to the Principal Physicist. Salary will depend on qualifications and experience.

Applicants should write, stating age, qualifications, experience and current salary, and giving the names of two referees, to the House Governor, Charing Cross Hospital, London, W.C.2, not later than October 16, 1965.

UNIVERSITY OF SOUTHAMPTON

CHAIR OF BOTANY

Applications are invited for the Chair of Botany. The appointment will date from January, 1966 (or later by arrangement). Salary within the normal professorial range of £3,400 to £4,750 per annum. F.S.S.U.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton, to whom applications (fourteen copies from applicants in the United Kingdom), should be sent before October 30, 1965.

FOURAH BAY COLLEGE

THE UNIVERSITY COLLEGE OF SIERRA LEONE

Applications are invited for Lectureship in Geology. Salary scale: £1,200 to £2,370 per annum. F.S.S.U. Children's, car, and outfit allowances. Family passages. Biennial overseas leave. Part-furnished accommodation.

Detailed applications (six copies), naming three referees, by October 28, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

APPLICATIONS ARE INVITED FOR RESEARCH Assistantships in Radiochemical Studies. Persons appointed will be expected to register for the Internal M.Sc. or Ph.D. degrees of the University of London. They should possess a degree in chemistry or equivalent, and preferably be interested in the kinetics of exchange reactions. Salary £750 per annum in the first year. Applications, together with names of two referees, should be sent direct to the Senior Lecturer in Radiochemistry, Sir John Cass College, Jewry Street, London, E.C.3.

UNIVERSITY OF SOUTHAMPTON

DEPARTMENTAL ADMINISTRATOR

Applications are invited for this new post in the Department of Chemistry. Salary scale £1,400 by £85 to £2,505 per annum, with a merit bar at £2,165. F.S.S.U. benefits. Starting salary according to qualifications and experience. Applicants should have a degree or equivalent.

The Departmental Administrator will be responsible for the organization and administration of a complex of buildings, at present housing some 100 staff and research workers, and about 200 undergraduates. He will be a member of the academic staff and will be expected to play a full part in the life of the Department.

Applications (seven copies), giving date of birth, a brief curriculum vitae, and the names of two referees, should be sent to the Deputy Secretary, The University, Southampton, from whom further details may be obtained, before November 6, 1965.

FIELD TECHNICAL OFFICER

(AGRONOMIST)

Applications are invited from those suitably qualified in agriculture and/or agricultural chemistry, for the post of Field Technical Officer. The successful candidate will be responsible to the Chief Technical Adviser for the carrying out of field experiments with the Company's products and will assist in providing technical advice to both sales staff and farmers. Knowledge, and preferably experience, of field experimentation is essential. The appointee will be based at the Company's Headquarters at Hartham, Corsham, Wilts, but his duties will take him over the whole of the Company's trading area and a car will be provided.

Applications, giving full details of qualifications and experience, should be addressed to the Chief Technical Adviser, Soil Fertility Ltd., Hartham, Corsham, Wilts., to arrive not later than two weeks from the date of this advertisement.

UNIVERSITY OF LIVERPOOL

Applications are invited for three posts of Assistant Lecturer or Lecturer in the Department of Physics. The initial salaries will be within the range £1,050 to £2,505 per annum, according to age, qualifications and experience. Candidates should have research experience in nuclear structure or particle physics.

Applications, stating age, academic qualifications and experience, together with the names of three referees, should be received not later than November 27, 1965, by the Registrar, from whom further particulars may be obtained. Please quote Ref. CV/290/N.

SENIOR TECHNICIAN

A Senior Technician is required for the Ecology section of the Department of Forestry, Oxford, to assist in a programme of laboratory and field research. Candidates should have a Higher National Certificate (or equivalent qualification) in Physics, Chemistry or Biology. Preference will be given to those with experience in physical instrumentation and an interest in field work.

Applications to Dr. M. E. D. Poore, Department of Forestry, Commonwealth Forestry Institute, University of Oxford.

ASSISTANT BACTERIOLOGIST

The Fruit and Vegetable Canning and Quick Freezing Research Association require an Assistant Bacteriologist from October 1, 1965, or earlier. Candidates, preferably female, should have a University degree and/or practical experience in food microbiology.

Applications to Director, Research Station, Chipping Campden, Glos.

APPLICATIONS INVITED FOR POST OF chemist, or biologist with chemical interests, in grade Assistant Experimental Officer (£719 at 21, rising to £1,201) or Experimental Officer (£1,319 rising to £1,675) for work on aspects of mineral nutrition, particularly potassium and micronutrients. Some knowledge of chromatographic and radioactive isotope techniques advantageous. Qualifications: degree in science, horticulture, or agriculture, H.N.C. in chemistry, or equivalent. Salary grade and point on scale dependent on qualifications and experience. Contributory superannuation scheme.—Applications, with names and addresses of two referees, to be sent to the Secretary, National Vegetable Research Station, Wellesbourne, Warwick by October 15, 1965.

READING TECHNICAL COLLEGE

Grade B Assistant Lecturer required in Department of Science to teach Chemistry mainly to O.N.C. and G.C.E. courses with the possibility of teaching Inorganic Chemistry up to Part II Grad. R.I.C. level for person with suitable qualifications.

Application forms (to be returned as soon as possible), and further particulars obtainable from the Principal, Reading Technical College, King's Road, Reading.

ST. BARTHOLOMEW'S HOSPITAL

LONDON, E.C.1

Applications are invited for the appointment of Principal Grade Biochemist. Applicants must possess a higher degree in chemistry or biochemistry. Whitley Council salary scale. Further particulars can be obtained from the Chemical Pathologist.

Applications, in writing, to the Clerk to the Governors, quoting reference No. PT/836.

IMPERIAL COLLEGE

Senior Technician required for maintenance and operation of modern equipment in polymer characterization laboratory. Opportunity to assist in original research. Previous experience with polymer equipment would be an advantage. Salary in the range £840 to £1,040 plus London Weighting plus supplement for certain qualifications (male or female).

Applications, in writing, to the Departmental Superintendent, Chemistry Department, Imperial College, London, S.W.7.

NORTH STAFFORDSHIRE HOSPITAL MANAGEMENT COMMITTEE

NORTH STAFFORDSHIRE ROYAL INFIRMARY (511 BEDS), STOKE-ON-TRENT

Senior Laboratory Technician, Grade I or II, according to qualifications and experience, required in Laboratory of newly formed Metabolic Unit. Applicant should have wide experience in Biochemistry but Final Diploma of I.M.L.T. in Biochemistry not essential. Whitley Council salary scales and conditions of service.

Applications, with names of two referees, to Hospital Secretary, from whom full details may be obtained.

UNIVERSITY OF BRISTOL

Applications are invited for the post of Lecturer (Grade II) in Theoretical Chemistry tenable from January 1, 1966. Salary scale £1,400 to £2,505 per annum with superannuation benefit.

Further particulars may be had from the Registrar, University Senate House, Bristol, 2, to whom applications should be sent by October 20.

ROYAL MILITARY COLLEGE OF SCIENCE

OPPORTUNITY FOR RESEARCH

Research assistants required for work on resonances excited by microwaves in the plasma of a discharge column and on application of these resonances in the field of electronics. Interesting work; excellent facilities; pleasant surroundings; initiative and independence encouraged. Work suitable for publication and submission for higher degrees. Candidates should have a good degree in Physics or Electrical Engineering. Experience not essential but will secure a higher salary. Accommodation in a Hall of Residence, if single. Possibility of a house if married.

Please write for details in confidence, to: Professor M. Potok, Royal Military College of Science, Shrivenham, Near Swindon, Wilts.

ANALYST, JUNIOR OR ASSISTANT, required for Sedimentology Research Laboratory, Department of Geology, Whiteknights Park, for analysis of silicates and carbonates. Minimum qualification of G.C.E. "O" level chemistry. For the post of Assistant, preference will be given to applicants offering G.C.E. "A" level chemistry and, or, experience. Salary up to £840 per annum according to qualifications.—Apply, in writing to the Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

DEPARTMENT OF GENETICS, I.N.A. (Paris) requires a trained cytologist (Ph.D.) to study effects of mutagenic chemicals on plant chromosomes.—Inquiries should be addressed to Professor H. Heslot, Institut National Agronomique, 16 Rue Claude-Bernard, Paris 5, France.

ONE SENIOR TECHNICIAN AND TWO TECHNICIANS required for teaching in Biochemistry Department and for Research in Protein Biosyntheses. Previous experience in Biochemistry desirable. Salary within scale for Universities.—Apply in writing to Secretary, Ref.: 7520, King's College, Strand, W.C.2.

UNIVERSITY OF ALBERTA

DEPARTMENT OF CHEMISTRY

Applications are invited for the position of

PROFESSOR in Physical Chemistry

Candidates in the fields of Solid State, Structure of Liquids, Spectroscopy or Thermodynamics are favoured, but all applications will be considered. Starting salary according to qualifications, in the range $15,000 to in excess of $20,000. Facilities for research are excellent and generous support is obtained from Provincial and National sources. The Department has 30 members and is active in all the major divisions of chemistry.

Further particulars may be obtained from

Professor Harry E. Gunning, Head,
Department of Chemistry,
University of Alberta,
Edmonton, Alta., Canada.

UNIVERSITY OF MANITOBA

WINNIPEG, CANADA

COMPUTER CENTRE

Due to the acquisition of a large scale computer and the establishment of a Department of Computing Science several openings are available for computer programmers and for Academic Staff at professorial and lecturer levels. Salaries offered are competitive with Canadian industry. Some interesting computer research projects are being carried out including generalized programming, hospital automation on ■ real time basis, generalized compilers and discipline orientated computer languages. Staff are able to pursue their own research interests.

Interested candidates at all levels of experience are invited to send résumés and any relevant references to Professor B. A. Hodson, Director, Computer Centre, University of Manitoba, Winnipeg 19, Manitoba, as soon as possible. Interviews in England will be conducted in November and starting dates will be between April 1 and September 1, 1966. All replies will be treated confidentially.

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ZOOLOGY

ECOLOGIST FOR FOX RESEARCH

Applications are invited for the post of Research Fellow to work on the general ecology of the Fox for a period of three years, to take up duty as soon as possible. Applicants should hold a first- or second-class honours degree. Ecological training and previous research experience desirable. Aptitude for designing field equipment, acquaintance with rural life and pursuits and ability to drive a car are necessary. The study will involve work in remote areas. Salary in range £1,050 upwards with placing according to qualifications and experience. Superannuation (F.S.S.U.) and removal allowance.

Further particulars should be obtained from the Secretary, the University, Aberdeen, with whom applications (two copies), should be lodged by October 23, 1965.

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(UNIVERSITY OF LONDON)

HUNTER STREET, LONDON, W.C.1

DEMONSTRATOR (Honours B.Sc. Biochemistry or Chemistry) required to assist in laboratory and tutorial instruction in Department of Biochemistry. Opportunity to study for higher degree. Salary in range £700 o £1,000.

Applications, with names of two referees, to Secretary of the School as soon as possible.

TECHNICIAN REQUIRED FOR BIOchemical work including research, in University Department. Interesting work involving ■ variety of techniques. Whitley Council salary scales and conditions of service; salary according to age, experience and qualifications.—Application forms from the Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5. (Ref. B.) (M).

UNIVERSITY OF LONDON

CHAIR OF HAEMATOLOGY AT ST. BARTHOLOMEW'S HOSPITAL MEDICAL COLLEGE

The Senate invite applications for the Chair of Haematology tenable at St. Bartholomew's Hospital Medical College (salary in the range £3,645 to £4,445 a year).

Applications (ten copies), must be received not later than November 1, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

The proposed
UNIVERSITY OF BATH
A TECHNOLOGICAL UNIVERSITY
(Bristol College of Science and Technology)

RESEARCH ASSISTANT

Applications are invited from Physicists and Chemists for the post of Research Assistant in the Centre for Nuclear Studies. Candidates should have a degree or equivalent qualification. The successful applicant will carry out research and development work in radiometric and radioactivation analysis and will assist in the organization and maintenance of ■ radio isotope measurements laboratory.

The appointment is for two or three years in the first instance. Salary in the range £760 to £1,020 per annum.

Further particulars and application form from the Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7.
Please quote reference No. CST 65/96.
Closing date November 8, 1965.

UNIVERSITY OF LEEDS

Applications are invited for an algologist to collaborate as research assistant in an investigation of the polysaccharides in seaweeds. Applicants should have postgraduate experience preferably including the culturing of algae. The position is offered for two years in the first instance. Technical assistance in new accommodation will be provided specifically for these researches. Salary in the range £900 to £1,100 depending upon ■■ and experience.

Applications, with the names of two referees, should be made to Professor R. D. Preston, F.R.S., from whom further details may be obtained.

ISOTOPE TECHNICIAN FOR BUSY Laboratory using radioactive isotopes of iodine for investigating and treating thyroid disorders. Knowledge of Biochemistry or M.S.R. desirable, not essential.—Apply, Hospital Secretary, New End Hospital, London, N.W.3.

RHODESIA GOVERNMENT

MINISTRY OF AGRICULTURE

TEA SPECIALIST

A vacancy exists for a professionally qualified and experienced Tea Specialist to provide tea producers with technical advice on their production problems, to keep in touch with tea research in other countries and to co-operate with other specialists (plant pathologist, entomologists, soil chemists, horticulturists, etc.) in promoting the welfare of the Rhodesian tea industry. The Tea Specialist would also be expected to carry out some investigational development of tea research in Rhodesia if this becomes necessary. The Tea Specialist would be based in the modern town of Umtali, but would be required to travel extensively in the course of his duties. The Rhodesian tea industry is, at present, in the hands of four companies with over 6,000 acres planted and a production of nearly 4 million lbs., but this is expected to double when in full bearing; furthermore, there is also considerable scope for expansion in tea production by estates and by settlement schemes. Employment will be on ■ two to four year contract at ■ salary to be negotiated.

Application forms and further details from the Public Service Attache, Rhodesia House, 429 Strand, London, W.C.2.

UNIVERSITY OF LONDON

WELLCOME CHAIR OF PHARMACOLOGY AT SCHOOL OF PHARMACY

The Senate invite applications for the Wellcome Chair of Pharmacology tenable at the School of Pharmacy (salary not less than £3,400 a year plus £100 London Allowance). The successful candidate who will be Head of the Pharmacology Department in the School should be able to take up duties on October 1, 1966, or as soon as possible thereafter.

Applications (ten copies), must be received not later than October 28, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF ST. ANDREWS

Applications are invited for ■ Post Doctoral Assistantship (£1,050 by £75 to £1,200 per annum) and a Technical Assistantship (salary in the range £600 to £840 per annum) in Inorganic Chemistry at St. Salvator's College, St. Andrews. Successful applicants will work under the supervision of Dr. J. F. Nixon. The technical assistant will not be eligible for registration for a higher degree, and the appointment will be for one or two years with the possibility of permanent employment later.

Further information from, and applications, with the name of one referee, to the Secretary, Department of Chemistry, St. Salvator's College, St. Andrews, Fife, as soon as possible.

ST. BARTHOLOMEW'S HOSPITAL AND MEDICAL COLLEGE

Assistant (non-graduate; preferably male) for Medical Professorial Unit for biochemical research involving automated amino acid analysis. Salary in accordance with experience and qualifications on scale £880 to £1,100.

Apply, in writing, to the Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 611, reference No. PT/856.

TECHNICIAN OR JUNIOR TECHNICIAN required for Anatomy Department mainly for histology and photography in association with electron microscopy. Salary within the scale—Technician: £850 to £1,080; Junior: £525 to £790.—Apply Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

THE UNIVERSITY OF LEEDS: APPLICAtions are invited for the post of Technician in the Electron Microscope Laboratory, The Procter Department of Food and Leather Science. An experienced person able to operate and maintain the instrument and to assist in the preparation of biological specimens is preferred, but an applicant willing to be trained will be considered. Salary according to experience and qualifications.—Applications to Professor A. G. Ward, Procter Department of Food and Leather Science, The University, Leeds, 2.

HOSPITAL BIOLOGIST OR JUNIOR BIOchemist for duties involving biological assay and some hospital biochemistry. Opportunities for research and to obtain higher qualifications. Whitley terms.—Applications to Deputy Director, Vincent Square Laboratories of Westminster Hospital, 124 Vauxhall Bridge Road, London, S.W.1.

UNIVERSITY OF LONDON KING'S COLLEGE

LECTURER IN BIOCHEMISTRY

The College will require on or after January 1, 1966, a Lecturer in Biochemistry.

Salary scale: £1,805 rising by increments of £100 to £2,505 plus £60 London Allowance.

Conditions of appointment and application forms may be obtained from the Registrar, King's College, Strand, London, W.C.2; completed forms must reach him by October 31.

FOREST ENTOMOLOGIST

EAST AFRICAN COMMON SERVICES ORGANISATION

Required to work under the general direction of the Head of the Forest Entomology Division and the Director of the East African Agriculture and Forestry Research Organisation on extensive field studies of tree-borers and defoliators, to assist with the East African Forest Insect Survey and with the expansion and upkeep of the E.A.A.F.R.O. reference collection of forest insects.

Candidates must have ■ degree in Zoology or Entomology, with postgraduate experience in some aspect of tropical forest entomology.

Salary scale, £1,221 to £2,097 a year plus 25 per cent terminal gratuity. A general increase of 12½ per cent on existing salaries has been approved, but precise details are not yet available. Passages provided. Quarters available. Generous home leave. Two-year contract. Education allowances.

Candidates (under 45) who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name, and brief particulars of qualifications and experience, quoting RC 324/214/02 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the position of Head Technician in the Department of Medical Chemistry of the John Curtin School of Medical Research. The Head Technician is responsible to the Head of the Department, for the general supervision of a technical staff numbering 20, the provision of chemicals and apparatus, general administrative duties, staff training, and the supervision of all services to the laboratories. The Department is concerned entirely with research mainly in heterocyclic and organo-metallic-complex chemistry, and is equipped with instruments for many kinds of spectroscopic and potentiometric studies. Applicants should be familiar with these techniques and have ■ sound knowledge of chemistry based on a wide experience in laboratory practice. Salary offered is within the limits £A2,189 to £A2,924 per annum, but the top of the range will only be offered to an exceptional candidate who has formal qualifications. The University will pay reasonable removal expenses and provide assistance in obtaining housing. The position carries superannuation and long service leave benefits.

The successful applicant will be required to undergo ■ medical examination. Applications close with the undersigned at Box 4, G.P.O., Canberra, Australia, on October 30, 1965.

W. S. HAMILTON.
Bursar.

UNIVERSITY OF KENT AT CANTERBURY

CHAIR OF ORGANIC CHEMISTRY

Applications are invited for a Chair of Organic Chemistry, tenable from October 1, 1966. Further particulars may be obtained from the Registrar, University of Kent at Canterbury, Canterbury.

Applications (twelve copies, except for overseas candidates who need submit only one), should be received not later than October 30, 1965.

UNIVERSITY OF GLASGOW

PROGRAMMING ASSISTANT IN THE COMPUTING DEPARTMENT

Applications are invited for the post of Programming Assistant in the Computing Department from November 15, 1965. The Assistant would be required to write programmes for the University's KDF 9 computer and to assist in the general supervisory service of the Department. The appointment is on ■ scale of £800 to £1,275. Opportunity of study for ■ research degree (M.Sc. or Ph.D.) can be provided.

Applications, three copies, with the names of three referees, should be lodged, not later than October 15, 1965, with the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF MATHEMATICS COMPUTATION LABORATORY

Applications are invited for the post of Lecture or Assistant Lecturer. Candidates should have research experience in any branch of computer science but an interest in programming languages, systems programming or non-numerical applications of computers would be an advantage. An I.C.T. 1907 Computer System is on order for the Spring of 1967 but an I.C.T. 1909 will be installed in the Spring of 1966. Salary scales: Lecturer, £1,400 by £85 to £2,505 with ■ merit bar at £2,165; Assistant Lecturer, £1,050 by £75 to £1,275. The initial salary will depend on qualifications and experience.

Further particulars should be obtained from the Deputy Secretary, the University, Southampton, to whom applications (seven copies from United Kingdom applicants), should be sent not later than October 22, 1965.

ST. BARTHOLOMEW'S HOSPITAL

LONDON E.C.1

Technician in Department of Obstetrics and Gynaecology for research work in Cytology. Someone with previous experience preferred but a trainee would be considered. Salary scale within the range £415 to £1,100 according to experience.

Write, naming two referees, to the Clerk to the Governors, quoting Project No. 849, reference No. PT/854.

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LECTURES AND COURSES

WEST HAM COLLEGE OF TECHNOLOGY

PHYSICS DEPARTMENT
SESSION 1965/6

A course of evening postgraduate lectures in EQUILIBRIUM AND NON-EQUILIBRIUM STATISTICAL PHYSICS commencing Wednesday, October 13 at 6.30 p.m.
Lecturers: Professor R. K. Eisenschitz, Dr. J. R. Miles, Dr. J. Manuel

Topics will include: Fundamentals of classical and quantum statistical mechanics and applications to solid, liquid and gaseous systems; stochastic processes; transport processes in liquids and gases; fluctuation dissipation theorems; general theories of irreversible statistical mechanics. The course is designed to lead to the M.Sc. degree of London University by examination after one year and dissertation after ■ further year.

For more detailed information please apply to: Dr. J. R. Miles, Physics Department, West Ham College of Technology, Romford Road, London, E.15.

WEST HAM COLLEGE OF TECHNOLOGY

PHYSICS DEPARTMENT
SESSION 1965/6

A course of evening postgraduate lectures in NUCLEAR PHYSICS commencing Thursday, October 14 at 6.30 p.m.
Lecturers: Dr. A. Mason, Dr. P. Kalmus, Dr. L. R. Day, Dr. W. K. Mansfield, Mr. L. D. Brown, Dr. J. Shaw

Topics will include basic nuclear physics, nuclear forces, nuclear models, reactions, fission, detection, acceleration, neutron physics, cosmic rays, reactor physics, fundamental particles, radiation physics, and there will be opportunity for practical work. The course is designed to lead to the M.Sc. degree of London University by examination after one year and dissertation after ■ further year.

For more detailed information please apply to Dr. L. R. Day, Physics Department, West Ham College of Technology, Romford Road, London, E.15.

THE MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY

(Faculty of Technology in the University of Manchester)

A course of nine evening lectures on SOME RECENT ADVANCES IN THE CHEMISTRY OF THE TYPICAL ELEMENTS will be held at the College on Fridays commencing on October 8

Further particulars can be obtained from the Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester, 1.

LANCHESTER COLLEGE OF TECHNOLOGY

COVENTRY

COURSE ON INSTRUMENTAL METHODS OF ANALYSIS

A short course for analytical chemists will be held on Thursday, November 4 and Friday, November 5, 1965. Course Fee: £3 3s.

Details and application forms obtainable from the Head of the Chemistry and Metallurgy Department.

UNIVERSITY OF LONDON: A COURSE of two lectures entitled "The cell of the photosynthetic bacteria in light- and dark-metabolism" will be delivered by Professor G. Drews (Freiburg) at 5.30 p.m. on October 11 and 13 at University College (Botany Theatre), Gower Street, W.C.1. Admission free, without ticket.—James Henderson, Academic Registrar.

TECHNIQUES OF CRYSTAL GROWING

A three day course commencing Wednesday, November 10, 1965.

DIVISION OF MATERIALS AND MOLECULAR SCIENCE

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Contributors include specialists from Imperial College, Mullard Research Laboratories, R.R.E., University College, Hull University and Oxford University.

The course consists of lectures, short research contributions, laboratory experiments and demonstrations of equipment.

FEE: £7 (inclusive).

Details and application forms available from the Secretary, Woolwich Polytechnic, Wellington Street, Woolwich, S.E.18.

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF LONDON KING'S COLLEGE

RESEARCH STUDENTSHIP IN METALLURGY

MASS-TRANSFER IN SODIUM SYSTEMS

Applications are invited for a Research Studentship in Metallurgy. The work will be concerned with the mass-transfer of alloying elements, especially carbon, between plain carbon and alloy steels, immersed in high purity molten sodium at temperatures up to 850° C. Experiments will be made both under isothermal conditions, and with a temperature gradient existing in the sodium.

The value of the Studentship will be equivalent to the current S.R.C. rates, i.e., £500 per annum. Applications should be sent to the Registrar, King's College, Strand, London, W.C.2, not later than November 1, 1965, giving age, qualifications, experience and the names of two referees.

UNIVERSITY OF ADELAIDE

GWENDOLYN MITCHELL FOUNDATION FOR MEDICAL RESEARCH

Applications are invited from registered medical practitioners for appointment as

RESEARCH FELLOW

under the Foundation.

The Fellow will be required to conduct research in the field of Experimental Medicine. The status and salary of the Fellow will be those of a Senior Lecturer (salary range £A3,250 by £A110 to £A3,800) or a Reader (salary rate £A4,300) in the University, according to the qualifications and experience of the successful candidate. Superannuation on the F.S.S.U. basis will be provided on the basic salary; and an additional allowance of £A150 ■ year for a Senior Lecturer or £A200 a year for a Reader will be paid.

Prospective candidates are invited to obtain from the Registrar of the University or from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, a statement prepared for the information of candidates and a copy of the general conditions of appointment of subprofessorial staff in the University. Applications, in duplicate and giving the particulars specified in the statement, must reach the Registrar of the University not later than November 15, 1965.

IRELAND

RESEARCH FELLOWSHIPS IN FISHERIES SCIENCE

DEPARTMENT OF AGRICULTURE AND FISHERIES

Applications are invited from University Graduates for two Junior Research Fellowships in Fisheries Science relating to ■ research project into the water purity and related conditions affecting the fishing productivity of certain Irish rivers.

THE FELLOWSHIPS WILL BE TENABLE FOR 3 YEARS WITH A POSSIBILITY OF EXTENSION

INITIAL STIPENDS OVER £1,000 ANNUALLY MAY BE AWARDED TO SUCCESSFUL APPLICANTS SUBJECT TO THEIR HAVING HIGHER ACADEMIC QUALIFICATIONS AND/OR SUITABLE PREVIOUS RESEARCH EXPERIENCE.

Travelling and subsistence expenses will be at appropriate current Civil Service rates.

SPECIALIZED POST-GRADUATE TRAINING WILL, IF NECESSARY, BE MADE AVAILABLE FOR THE SUCCESSFUL APPLICANTS BEFORE THEY COMMENCE WORK ON THE RESEARCH PROJECT.

Applicants (men or women) should be of good standing in their academic sphere and must produce evidence of their qualifications. Higher University degrees—preferably in Biology and/or previous research experience in a field of science relevant to the research project are desirable but not essential qualifications. Such qualifications may, on evaluation, merit remuneration above the minimum of the scales. Application forms, further information on the Fellowships and conditions governing the awards can be obtained from the Fisheries Division, Department of Agriculture and Fisheries, 3 Cathal Brugha Street, Dublin, 1. (Telephone: Dublin 41121).

CLOSING DATE FOR THE RECEIPT OF APPLICATIONS WILL BE 5 p.m. FRIDAY, NOVEMBER 5, 1965.

J. C. Nagle,
Secretary.

Department of Agriculture
and Fisheries,
Dublin 1.

AUSTRALIAN INSTITUTE OF NUCLEAR SCIENCE AND ENGINEERING

POST-DOCTORAL FELLOWSHIPS

A small number of Post-Doctoral Fellowships may be awarded by the Institute to persons holding the degree of Doctor of Philosophy or equivalent who wish to undertake research projects within the fields of nuclear science and engineering.

Fellowships may be held directly with the Institute at Lucas Heights, near Sydney, N.S.W., or at any Australian University, preference being given to candidates whose projects require the use of facilities at the Research Establishment of the Australian Atomic Energy Commission, Lucas Heights. Initial tenure is two years, commencing normally from the date a Fellow begins full-time work on his project in Australia, and extension for ■ third year may be granted. Emolument will be not less than £A2,300 per annum, and not more than £A3,500 per annum. Emolument is liable to Australian income tax. Funds for equipment and other expenses may be provided. In the case of a candidate residing overseas at the time he accepts a Fellowship, the Institute may, under certain conditions, meet all or part of the cost of travel to Australia for ■ Fellow and his family.

Nominations or direct applications, as appropriate, will be received by the Institute at any time. Inquiries should be addressed to the undersigned at the Australian Institute of Nuclear Science and Engineering, Private Mail Bag, Post Office, Sutherland, N.S.W., Australia.

E. A. PALMER,
Scientific Secretary.

UNIVERSITY OF LEICESTER

RESEARCH FELLOWSHIPS IN CHEMISTRY

Applications are invited for post-doctoral Research Fellowships in Organic Chemistry for work under Professor C. W. Rees and Dr. B. Capon. Salary £1,050 to £1,250 per annum, according to qualifications and experience.

Applications should be sent to the Registrar as soon as possible.

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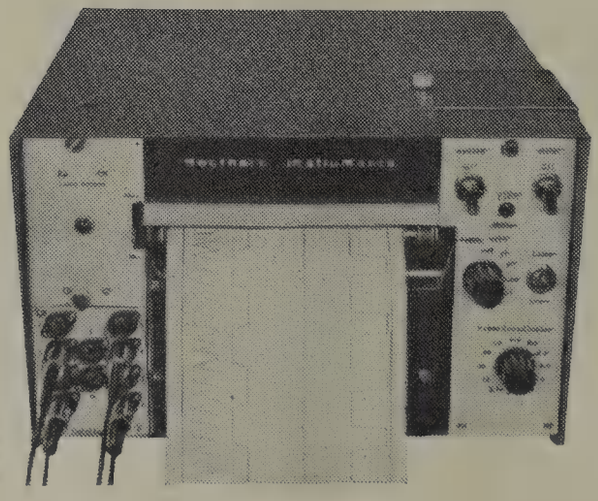

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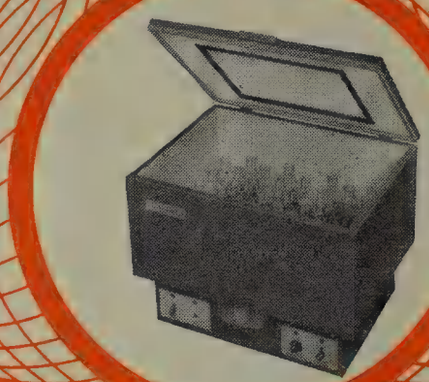

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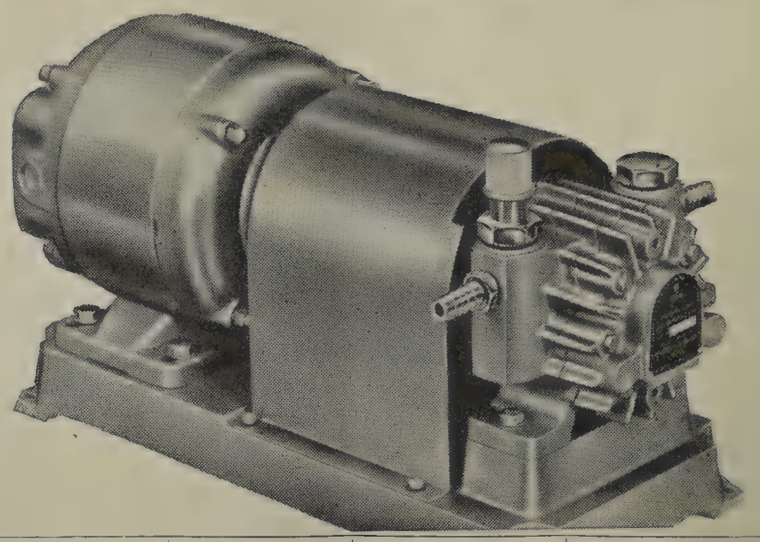

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L Phenylalanine
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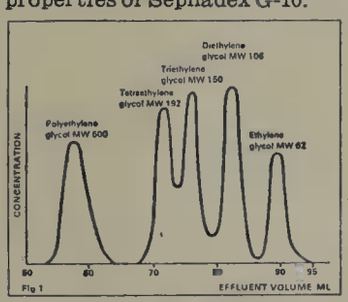
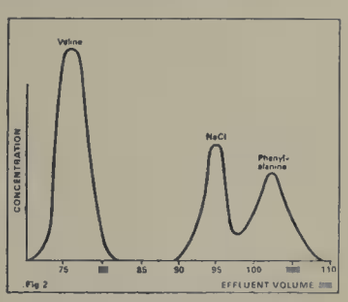


Fractionation of oligomers of ethylene glycol on Sephadex G-10.



Separation of valine, NaCl and phenylalanine on Sephadex G-10.

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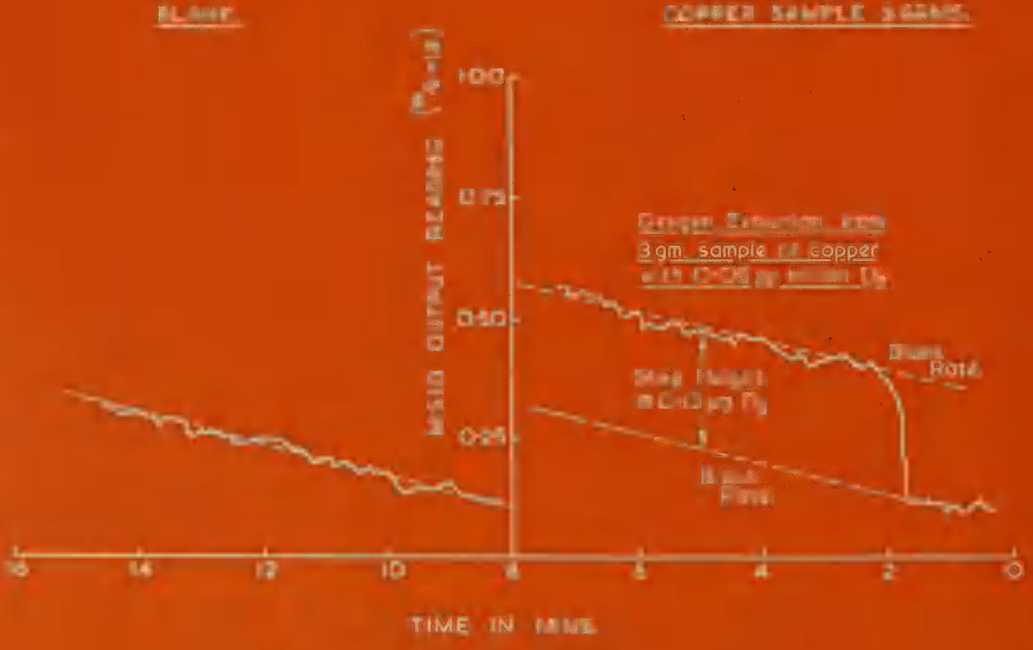
MS10

Ask for publication TP10, which gives full details of this technique (only one of the many MS10 applications) from A. J. Bullough, Associated Electrical Industries Ltd., **Scientific Apparatus Dept.,** Barton Dock Road, Urmston, Manchester. Tel (061) LONgford 4466. Ext. 371



AEI ELECTRONICS

WATSON news

HILUX BASE

for Bactil-60 and Service 3 microscopes with quartz iodine lamp

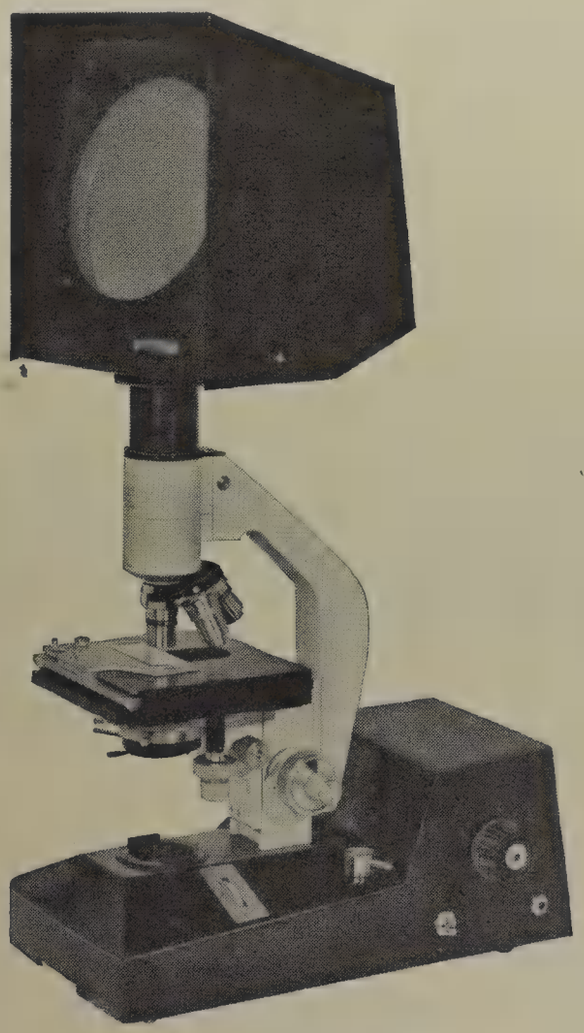
The new base brings to the Bactil-60 and Service 3 microscopes all the advantages of the quartz iodine lamp. The Hilux Base houses a carefully designed optical system to provide high quality Kohler illumination for all objectives. A simple switch introduces an auxiliary lens for the highest powers—no flip-top condenser needed — and the field iris control is handily located. Light intensity is continuously variable.

The level of illumination is ample for daylight screen viewing, projection, all forms of photomicrography and for phase contrast or fluorescence techniques. Easily adapted accessories are available for use with the Watson Hilux Base, including a rigid support for the Watson Polaroid Land Camera.

There are viewing screens for the Bactil-60 and Service 3 microscopes which present a well defined image under daylight conditions at all magnifications. Eyepieces are readily interchangeable and the screen can be rotated through 360°.

Projection eyepieces x2.5, x3.5, and x4.5 are available. Hilux Bases can be fitted to existing Bactil-60 and Service 3 microscopes.

Delivery early July. Please write for details and demonstration.

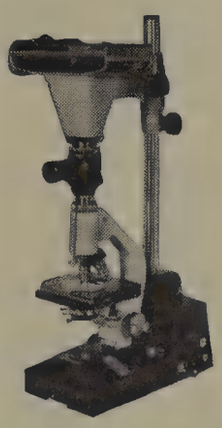


Bactil-60 microscope fitted with Hilux Base and viewing screen

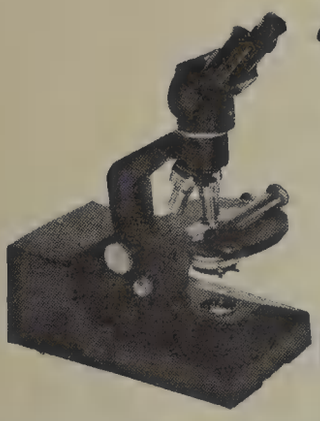
Service 3 with Hilux Base and viewing screen

Service 3: Hilux Base, projection eyepiece

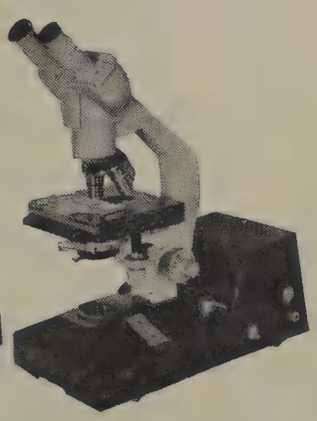
Bactil-60: Hilux Base, Polaroid Land Camera



Service 3 with binocular head, Hilux Base



Bactil-60 with binocular head, Hilux Base



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SCIENTIFIC APPARATUS
BULLETIN NO.7

MALARIA AND THE ELECTRON MICROSCOPE

Fig. 5 *Ookinete of* Plasmodium cynomolgi bustianellii *in the mid-gut of* A. stephensi. *The ookinete is in the process of penetrating the mosquito gut cell. Visible are the anterior slit, the mosquito gut cell and the brush border. (Magnification x 31,000).*

Until the introduction of the AEI EM6B, all commercially available electron microscopes were general purpose instruments rather better adapted to metallurgical and similar industrial applications than to biology. This mattered little so long as microbiologists were mainly concerned with morphological problems, but it became a disadvantage when interest shifted to the structure of viruses because it was not possible to reconcile versatility with the highest possible resolution. Now the biologist has in the EM6B an instrument custom-built to his particular needs. It is therefore an appropriate juncture at which to review the ways in which electron microscopy has contributed to the study of small organisms. Consider, therefore, its use in the study of malaria.

Blood forms

The electron microscope was first used in connection with malaria research in 1942 when Wolpers applied it to the study of blood forms. This initial essay did not prove wholly successful because the thickness of the thinnest sections obtainable with the techniques then available was too great for detailed observation. In due course, however, the method of cutting thin sections by means of the ultra-microtome was developed, and the electron microscope began to yield results.

In 1956 Fulton and Flewett proved that the trophozoite stages of *Plasmodium berghei* and *P. knowlesi* lie, not on the periphery of the ethrocytes but inside. This together with the work carried out by Garnham, Bird and Baker on *Lankesterella garnhami*, and by Maria Rudzinska and Trager on the presence of phagotrophic food vacuoles and mitochondria in *P. berghei* and *P. lophurae*, firmly established electron microscopy as a major technique for advanced work in this field.

Observation of sporozoites

The work of Garnham, Bird and Baker was particularly important since it led, in 1960, to the series of papers describing the fine structure of various malaria parasites. Three of these papers deal with forms of the parasite which are pathogenic to both avians and primates as they appear in the sporozoite stage. This is the long thin form of the organism which is injected into the bloodstream of the victim, via the salivary glands of the mosquito. Fig. 1 shows a longitudinal salivary gland containing many sporozoites in the acinar cells and just emerging from them. The sporozoite measures approximately 10 microns long and about 1 micron in width, these measurements varying slightly from one species to another.

In order to examine such specimens in the electron microscope an elaborate preparation technique must be used. After dissection from the mosquito, the specimens are immediately fixed in 1% osmium tetroxide solution buffered to pH 7. After fixation, the tissues are rapidly dehydrated through graded ethanol solutions up to absolute ethanol. 1% phosphotungistic acid is included in the third absolute ethanol change to stain the tissues, before a final rinse in ethanol.

The specimens are then embedded in Araldite, and sectioned on an ultra-microtome (each section being between 60 and 150 Angstroms in thickness) and subsequently examined in the electron microscope.

Constant features

Figures 2, 3 and 4 show typical sporozoites. *Haemameoba gallinacea* is responsible for malaria in birds—i.e. an avian form—while *Laverania falcipara* and *P. vivax* are both

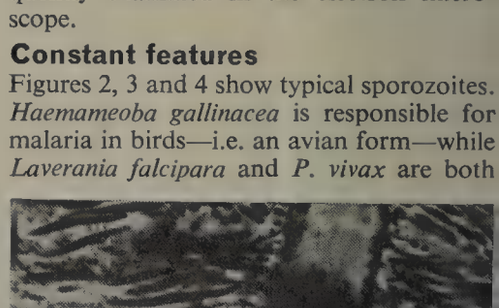
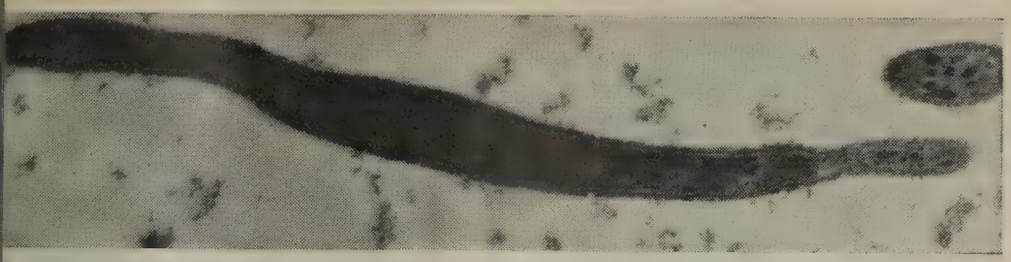


Fig. 1. *Longitudinal section of part of a salivary gland of* Anopheles gambiae *containing a large number of sporozoites of* Laverania falcipara *in the acinar cells and emerging into the salivary duct. (x 1,300).*

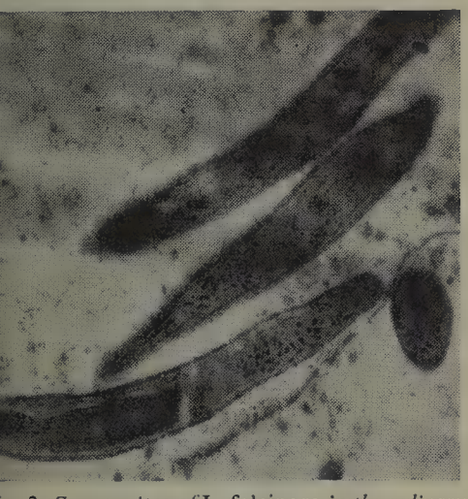


g. 2. *Sporozoite of* Haemamoeba gallinacea *the salivary glands of* Aedes aegypti queens-ndensis. *The longitudinal section is of the ddle region of the sporozoite and shows the nucleus, mitochondria and one lobe of the paired organelle. A transverse section also appears showing several mitochondria.* (*x 20,000*).

thogenic to primates. All three sporozoites ow the same basic structure, but minor fferences have been questioned. The oblems thus raised can only be resolved electron microscopy.

nstant features are the nucleus, mito-ondria, micropyle and thick pellicle. The cropyle is of particular interest since it is w thought to be a characteristic feature the sporozoites. The electron microscope s shown it to be a constant feature of all orozoites examined to date, and it has so been discerned in the sporozoan parasite *garnhami*. This account, brief though it is, early indicates the way in which the ctron microscope can be used in the entification of organisms. And since fine uctural details seem likely to prove the ly specific differences between organisms the kind under discussion the technique an important one.

ne basic principle is simple. Once con-ancy of appearance has been demonstrated may then be used as a positive index in e identification of a given organism. milarly, its absence is an indication that e organism does not belong to that genus. comparable exercise has been carried out the ookinetes of *Haemamoeba* and *lasmodium*. In this case the specimens ere stained in tungstophosphoric acid. A



ig. 3. *Sporozoites of* L. falcipara *in the salivary ands of* A. gambiae. *The sections are longi-dinal, a single transverse section being also resent, and show the nucleus apparently con-ected to the micropyle by a granular process nd mitochondria.* (*x 10,000*).

w minutes after the mosquito has ingested alaria infected blood, the micro and acrogamete fuse and produce the zygote. his body uncoils and lengthens to become ie ookinete.

he ookinete *H. gallinacae* has been studied detail by Garnham, Bird and Baker. nder the optical microscope the ookinete seen as a body about 15 microns in length, ith a large deeply staining nucleus (often ccentric in position), pigment granules nd a vacuole. The appearance under the electron microscope is shown in fig. 5. The nucleus has a patchy granular structure with no clearly defined nuclear membrane. The pigment granules are shown to be in the cytoplasm, appearing as irregular black masses lying in vacuoles limited by a membrane.

After developing for about 24 hours the ookinete is ready to pass through the mucosa of the mosquito's midgut. The process whereby this occurred was not discernable under the optical microscope and it was only comprehended when the electron microscope was applied to the problem.

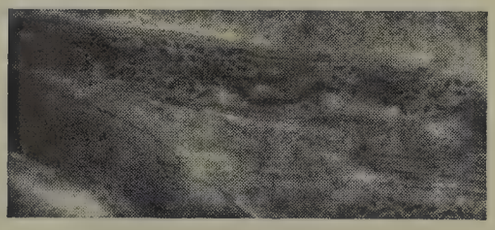


Fig. 4. *Sporozoites of* Plasmodium vivax *in the salivary glands of* A. stephensi. *It shows the paired organelle, micropyle, peripheral fibrils, convoluted tubules and the pellicle.* (*x 19,000*).

Biochemical studies

The fine structural details revealed by the electron microscope raised important questions as to the functions of the various organisms. Consequently research workers are becoming increasingly concerned with biochemical studies involving advanced techniques—such as enzyme localisation by radioactive marking—which call for very high resolution microscopy. The ultra high resolution of the EM6B Electron microscope makes it particularly suitable for work of this kind. It also simplifies more routine biological work, and for a number of reasons.

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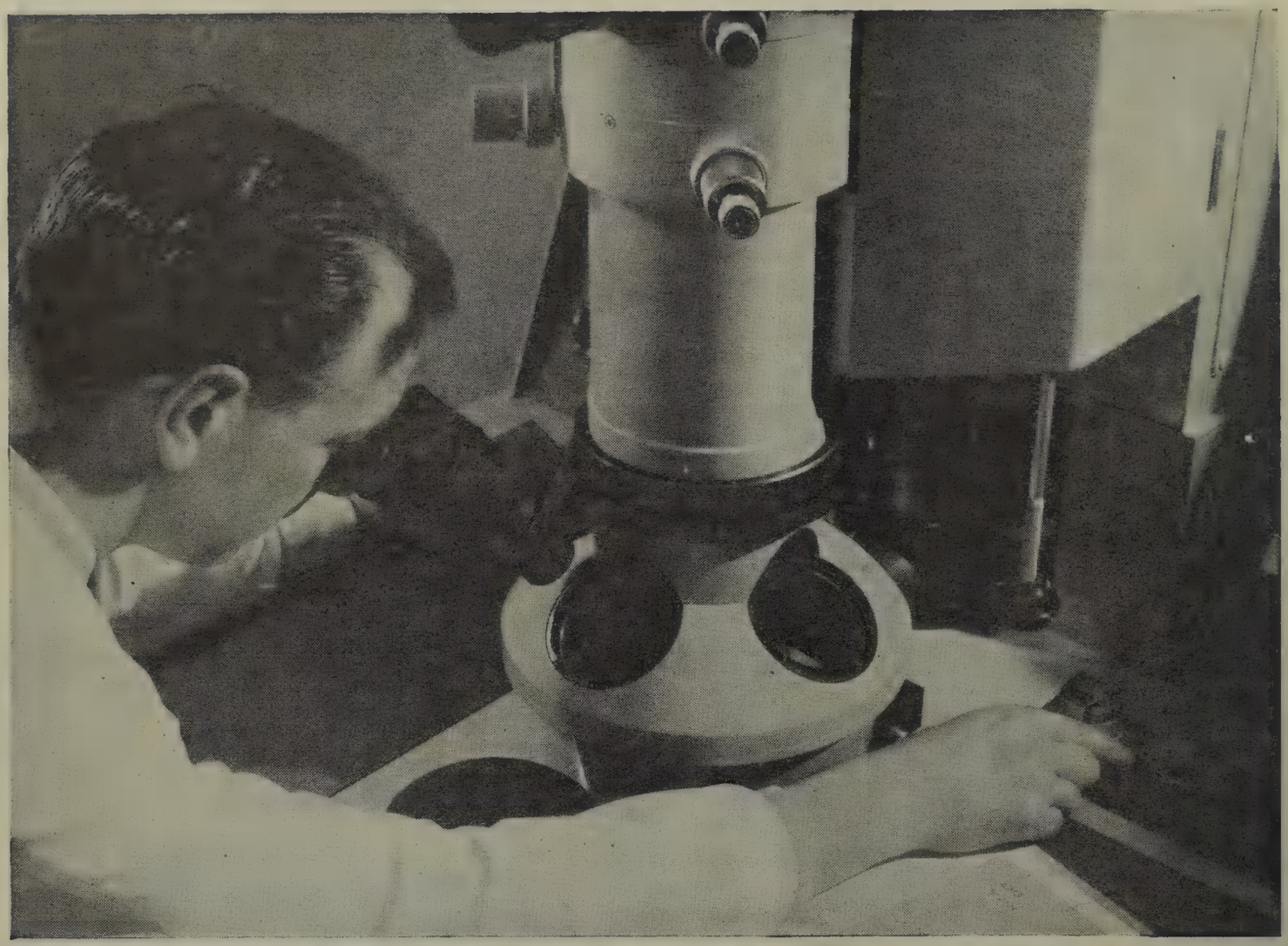

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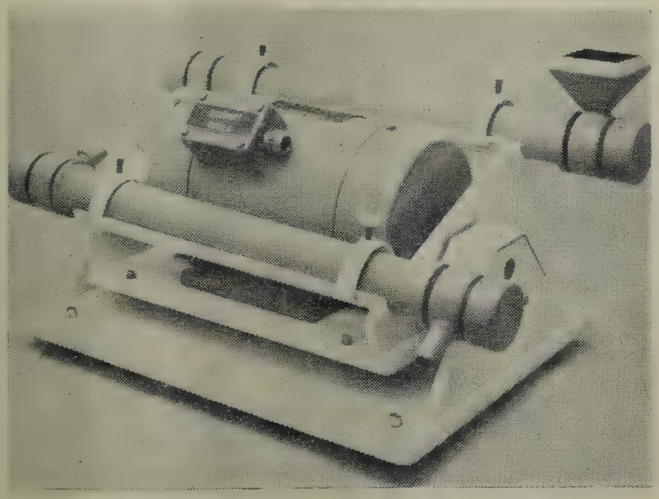

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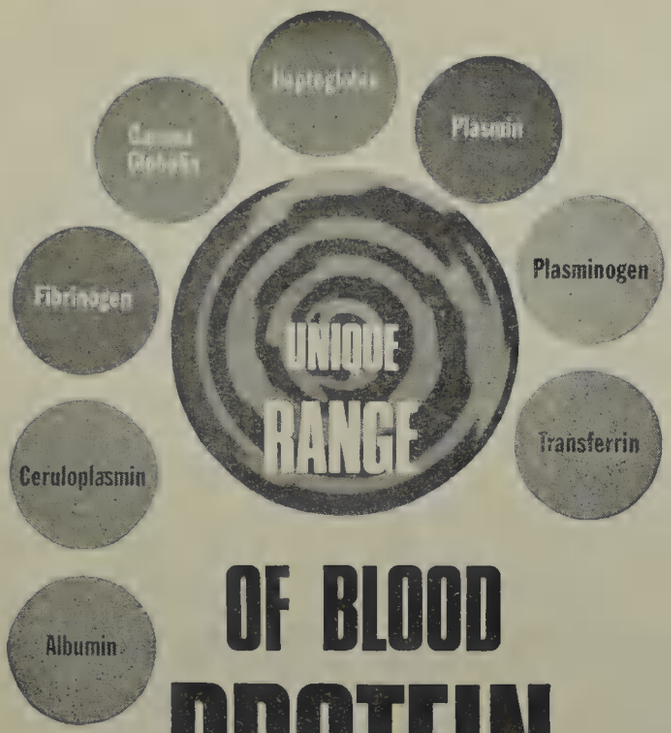

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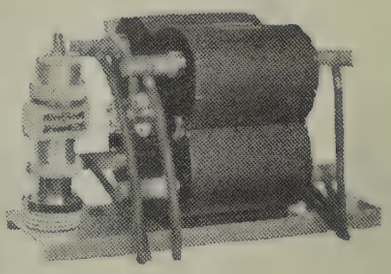

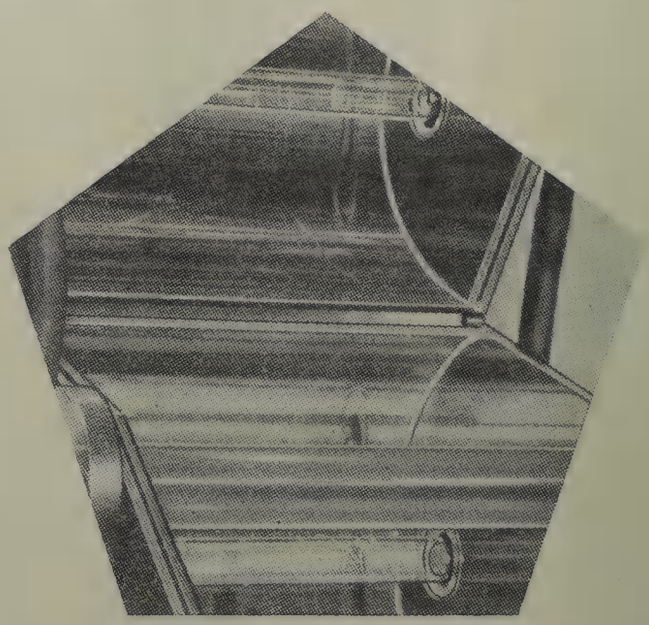

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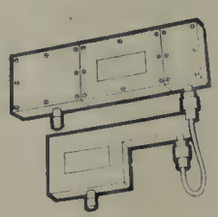

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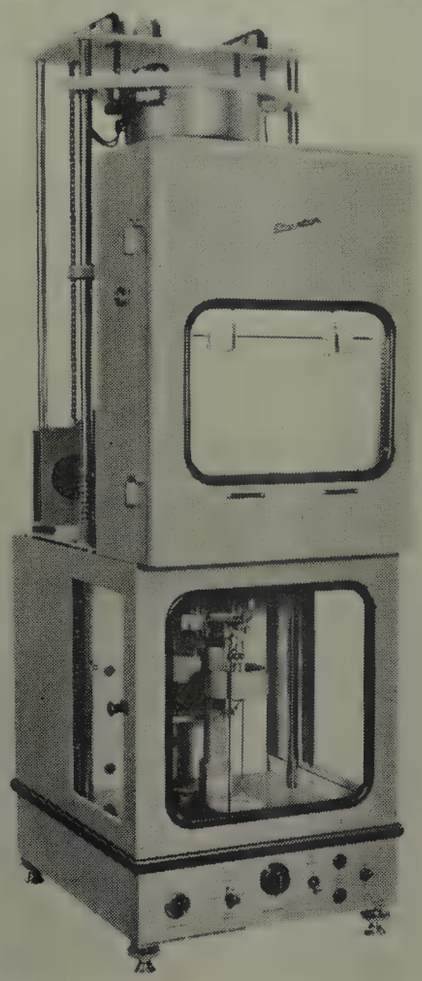

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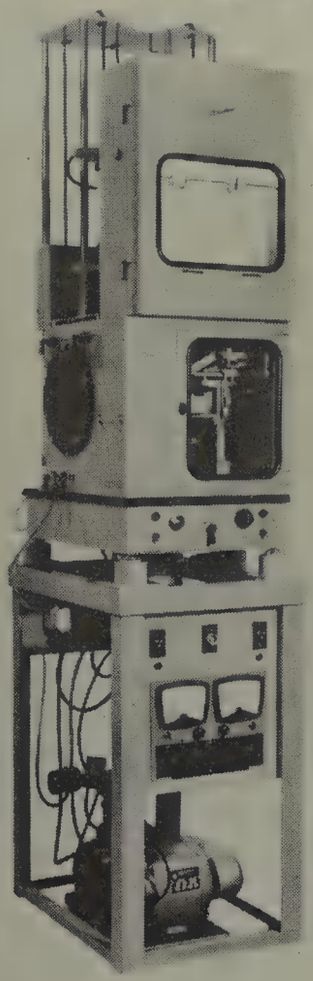

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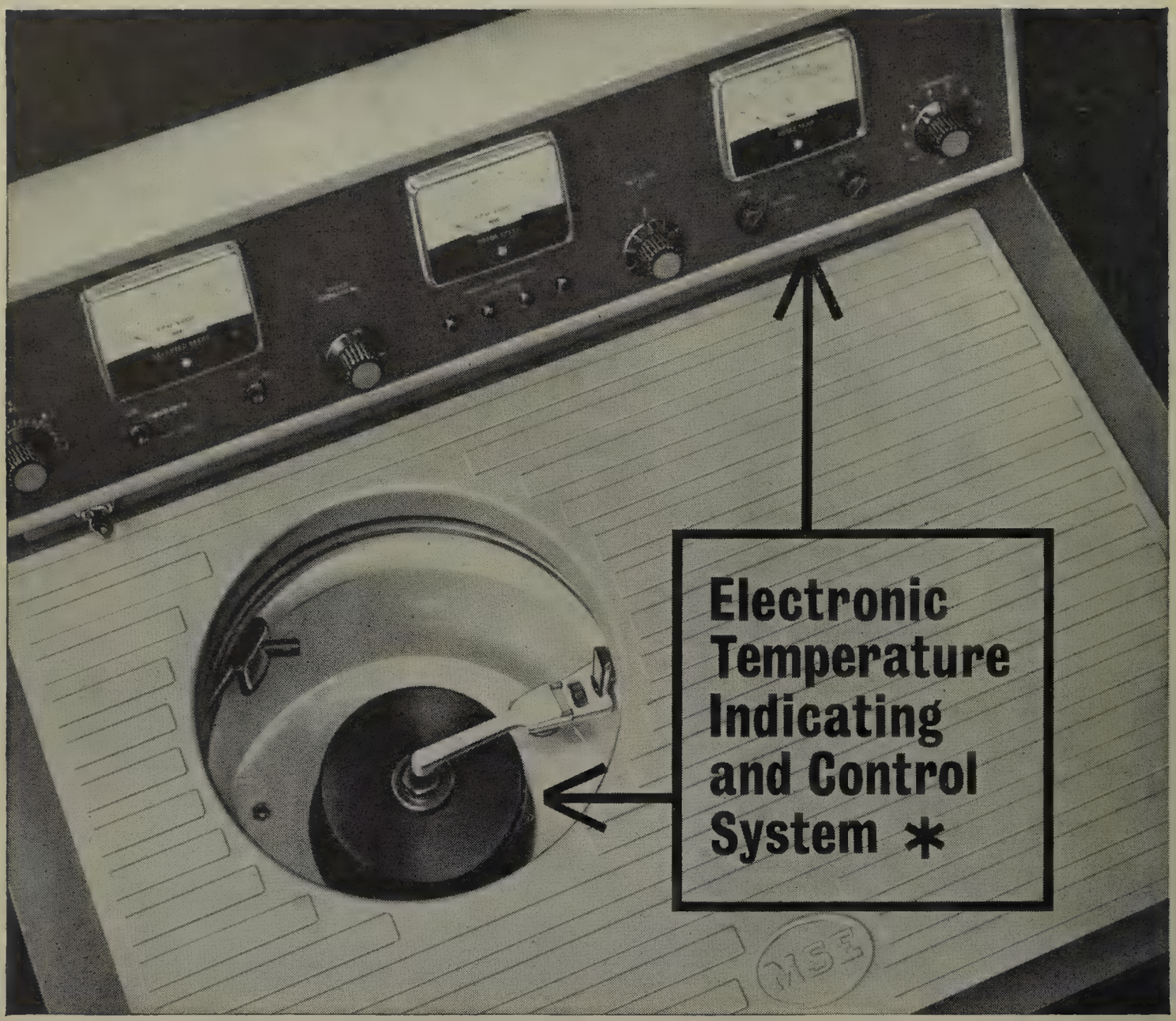

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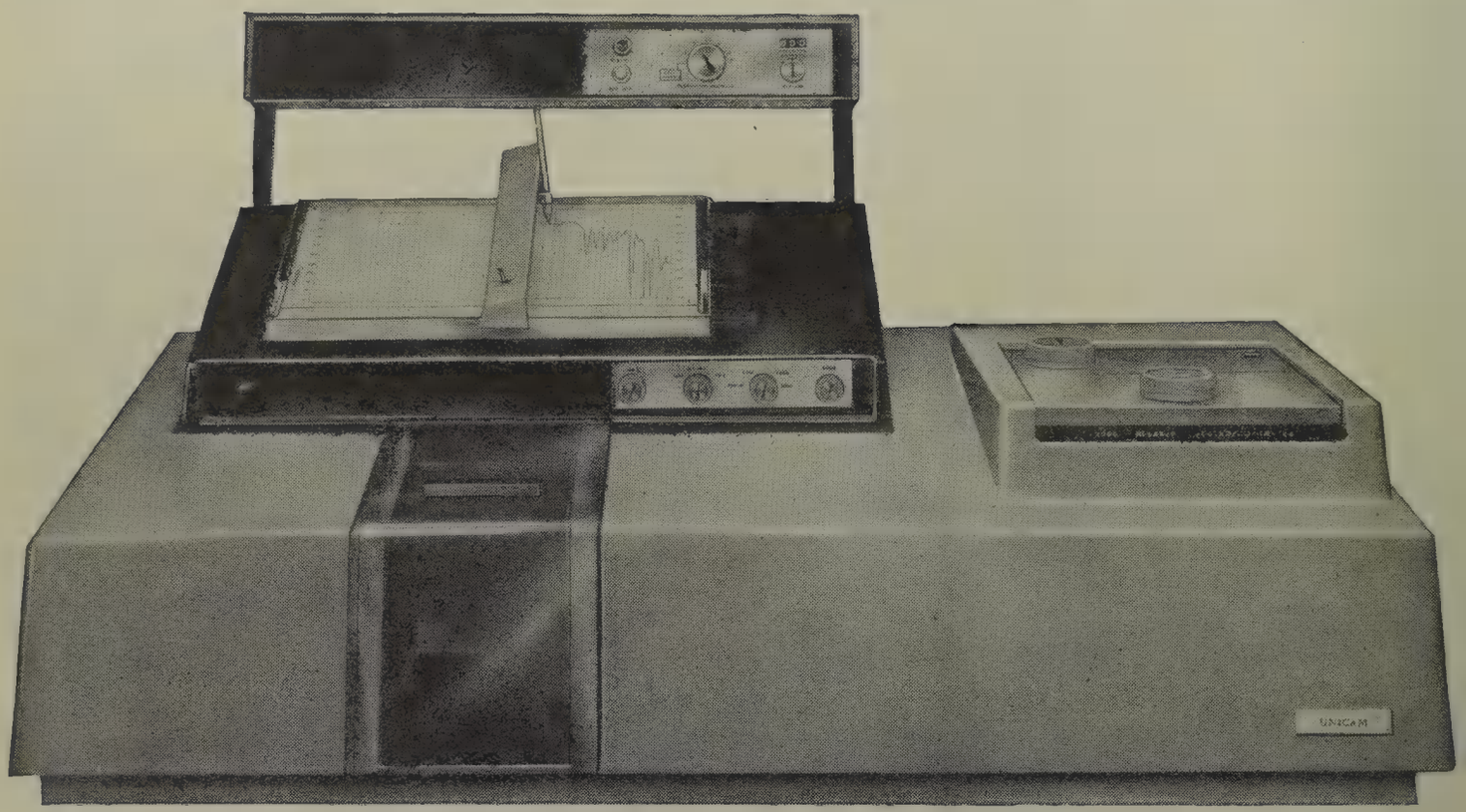

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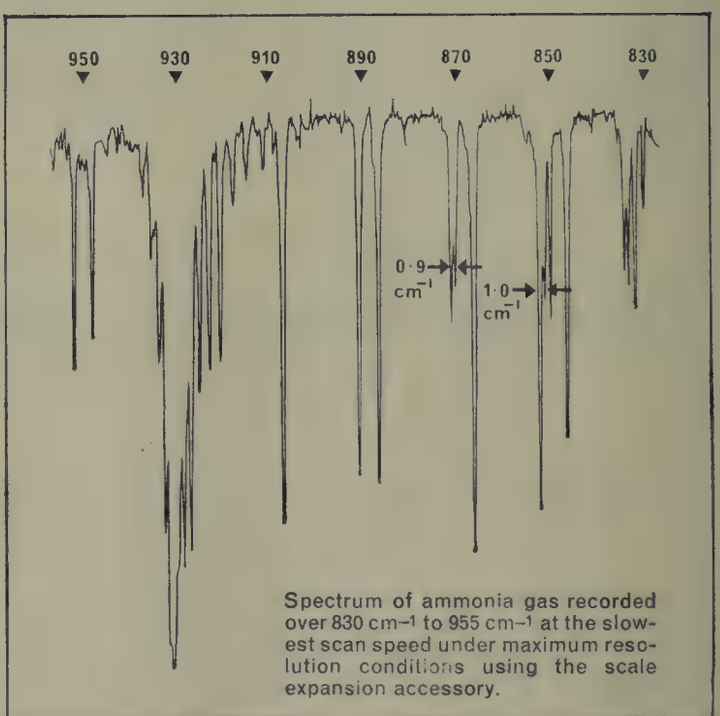

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RESEARCH AND DEVELOPMENT IN BRITISH INDUSTRY

PROF. J. W. MITCHELL'S stimulating Jubilee Memorial Lecture to the Society of Chemical Industry (*Nature*, 207, 113; 1965) on the organization of basic research for the British chemical industry was followed in May by Sir Ronald Holroyd's Brotherton Memorial Lecture to the same Society at Leeds, in which he discussed some aspects of research and development in that industry. More recently, at the annual general meeting of this Society in Cardiff in July, Mr. K. Piercy considered the broader picture of the chemical industry in a changing world, touching on such topics as the problems presented by technological change, research policy, logistics, long-range planning and the demands made on management and staff. Similar ground was covered by Dr. J. Taylor in a lively and provocative address entitled "Restrictive Practices", when he was awarded the Society's Medal at that meeting. Taken with Mr. J. C. Duckworth's eleventh Graham Clark Lecture to the Institution of Mechanical Engineers, "Incentives to Innovation and Invention", these lectures provide a highly stimulating and suggestive commentary on British industry in general and on the chemical industry in particular, which is worth examining in the context of the organization and activities of the Ministry of Technology and of Britain's economic situation generally.

Early in his address, Sir Ronald Holroyd, for example, points out that research in industry aimed primarily at commercial objectives has contributed greatly to the general body of scientific knowledge, and that such fields as colloid science, crystal-habit control and the chemistry of photography have developed very largely through industrial contributions. Such research work inside industry has called for the same degree of ingenuity and skill as any other form of research, and has involved more fundamental work than is commonly imagined. Indeed, the possibilities of conducting fundamental research in industry are considerably greater than is often appreciated, especially perhaps by teachers and others who may influence the choice of a career by a young graduate.

Sir Ronald is not, however, concerned with this point more than to emphasize the advantage which the greater resources of the industrial firm may confer in an integrated attack and the dependence of the future growth, diversification and productivity of chemical industry on the volume and efficiency of its own internal research effort. He is not unmindful, however, of the benefit derived from academic research or that in some fields Government institutions could provide valuable, if strictly limited, assistance. He is concerned, though, more especially with the way in which new discoveries and inventions in industry emerge: here his address runs parallel with that of Mr. Duckworth's.

Sir Ronald points out that the really successful company is one which, in addition to seeking new applications for existing knowledge, has the imagination to pick out potentially profitable objectives not obtainable with existing knowledge and the ability to work out an approach to these objectives through its own facilities for research and development. Success in identifying such objectives depends very largely on the early recognition of existing or future needs, whether in the company itself, in the industries it serves or in the community at large, and Sir Ronald adds that this responsibility must be spread over all branches of the company. The ordinary research scientist is not the most likely person to recognize or to assess the importance of such needs: his skill and inspiration are the dominant factors in seeking a scientific breakthrough towards a selected objective.

Accordingly, Sir Ronald reminds us that research, including technical development, cannot be treated in an industrial setting as an isolated function, but must be closely integrated with the other activities of the firm. Scientists in industry must recognize that they are a part of the overall management team and look to the practical and economic achievements of the company for their main personal satisfaction in their work. This he regards as the essential difference between research within and without industry, and it obviously has a close bearing on the ability of an industry to attract competent scientists. However, in his lecture, Sir Ronald stresses the responsibility it places on management for making effective use of its scientists, including the deliberate diversification of their expert experience. Neglect of this could be a potent factor in the shortcomings of the chemical industry which have led it to lose ground to foreign competitors to an extent for which the Economic Development Council could find no obvious explanation.

The Council did indeed suggest that uncertainty about the future growth of the economy was leading to hesitant investment, and that the high cost of British coal and electricity, as well as the comparatively low British tariff on the newer plastics, might be hidden obstacles. None of these, however, bears closely on the point discussed by Sir Ronald and by Mr. Duckworth, and it is worth noting that Mr. Duckworth, so far as the individual is concerned, discounts the primary importance of material incentives. Both here and in his comment that significant technological advances now tend to be made by teams rather than by individuals, his views support Sir Ronald's, at least by implication. He challenges the academic attitude towards patenting, as perhaps might be expected from the chairman of the National Research Development Corporation, and stoutly champions the policy of energetic filing and exploitation of patents.

Mr. Duckworth's views on industrial and national incentives are likewise largely derived from his experience with that Corporation. However, he points out that inventions and innovation are not necessarily meritorious in themselves but only so far as they contribute to higher efficiency and enable us to compete more effectively in world markets. While all significant technological information available in the United States is also available in Britain, the output per worker in the United States is in general 2·5–3 times that in British industry. Britain's immediate problems, he believes, are concerned mostly with management and with the attitude of workers to their jobs. He suggests that a thorough study of the reasons underlying this difference would be a most potentially rewarding research programme.

Mr. Duckworth again agrees with Sir Ronald when he remarks that, in the long run, achievement of a steady increase in productivity can be achieved only by the introduction of new processes and products. It is in this context that he suggests that a main purpose of taxation

should be to ensure, so far as possible, that individual firms, while looking after their own interests, act in the national interest also. Nevertheless, a recent survey has shown that when deciding whether or not to install a new process only 16 per cent of firms make prior allowance for all available tax incentives in their calculations. This may be an indictment of management but, as Mr. Duckworth observes, it makes it difficult to see what the next stage should be.

Mr. Duckworth sees four areas where direct Government action is, or would be, advisable in the national interest. First are those few projects of great significance and public interest where the research and development expenditure or capital cost involved are too great to be shouldered by an individual firm or groups of firms—as in the development of civil aircraft or nuclear power. It might be added that we have yet to elaborate really satisfactory procedures to provide the basis for even Government decisions in such major technological developments. Mr. Duckworth suggests that scientifically trained executives would make a great contribution in Government by ensuring that scientific method was applied in Government procedures so that the maximum possible information was available before decisions were taken.

Secondly, there is the analogous situation of a medium-sized project for which, as with the hovercraft, there is no particular industry in existence with the potential or expertise to develop it fully. The third area which he instances is concerned with the fact that a relatively long-term view is required as compared with the relatively short-term view which directors are usually compelled to take in the interests of their shareholders. The fourth is that where the estimated benefit to the innovating industry may provide insufficient incentive to proceed, although the benefit to the national economy as a whole may be considerable. Here a start has already been made with the use of cost/benefit analysis, and Mr. Duckworth concludes his lecture by stressing the vital part which the engineer or scientist who turns to management could play. It is to the application of scientific method and results to management and to new products and processes that we must look for increased productivity, though the creation of a society which welcomes innovation also depends largely on a re-orientation of our sense of values.

Much of what Mr. Duckworth says in this lecture is reflected in the closing part of Sir Ronald Holroyd's lecture, particularly in reference to management and the methods of assessments available or in use. Both are also conscious of the critical importance of recruitment, of ensuring that a due proportion of talent is attracted to engineering and technology and that the idea that industrial scientific activities are intellectually second-rate should be firmly dispelled. Mr. Piercy does not refer to this recruitment problem, but in referring to management and staff he stresses the exacting demands made on them in the chemical industry, with the result that their jobs are correspondingly more intellectually satisfying and interesting than in the past. He believes that the industry could do more to ensure that its able young men receive first-rate training in management at appropriate stages in their career and that, after giving them the opportunity to broaden their experience, the best use is made of their abilities.

Mr. Piercy's confidence in the ability and professional competence of such management in chemical industry is the more significant in that his address treats more specifically the policy and export performance of the industry which the Economic Development Council for the industry has since criticized. In referring to long-range planning, he does not mention the uncertainties stressed by the Council, but he admits that its export performance does not compare well with similar bodies in other countries. He is also concerned at the rise in chemical imports, for which he suggests no specific reason. Commenting on the trend towards larger plants, he believes that the risk of excess capacity has been over-exaggerated; while this trend makes the choice of a site more difficult, he agrees with Sir Paul Chambers as to the importance of really large markets.

On all this Dr. Taylor's lively address makes a most stimulating gloss. He puts the question of restrictive practices very fairly and pertinently in its proper context and in a way that indicates where the particular responsibility lies. Restrictive practices are, he points out, among the restrictions and inhibitions on our adaptability and rate of progress imposed by the illogical forces derived from man's past which dominate our behaviour. To recognize this is a first step toward eliminating the restrictive practices which, Dr. Taylor agrees, must be cut out if we really mean to improve our material prosperity. They are, in fact, widely present throughout our society and in so far as they do, as some argue, arise out of social and economic cleavages. Dr. Taylor considers that our troubles are due in greater part to a lack of leadership.

Dr. Taylor makes one point that is often overlooked in discussing our economic situation to-day: as a people, we made two all-out efforts in war in little more than thirty years, and whereas the defeated nations had help from outside in their subsequent struggle to survive, the British people have never had the opportunity to relax. It is equally important to note that Dr. Taylor does not commit himself that a materialistic objective is necessarily wise and this should be remembered when he emphasizes that British industry is more highly manned than its American counterpart and that only if the profit per person in productive industry is high can the standard of living in Britain be improved and capital resources built up. That the chemical industry is so highly manned should induce a careful study with the view of reducing manpower. To cut out restrictive practices is one way of achieving this, provided our diagnosis of those practices is accurate and complete, and takes account of those which arise from outside—more particularly from Government regulation or interference and tax systems.

Dr. Taylor faces the difficulties of this problem fairly, putting his finger on some of the real sources of friction, such as the differentiation between the salaried staff and the pay-roll, particularly in respect of redundancy. Without defending obsolete ideas and practices in the Trade Unions, he points out that their critics also sometimes fail to take account of changed conditions and that on the staff side too restrictive practices are to be found quite inconsistent with responsibilities which are the counterpart of their privileges. Moreover, if it is difficult enough for people of advanced education to adapt themselves to change and even the *tempo* of change, it is much more difficult for those who left school at the age of fourteen to make the transition from a well-established routine to fundamentally different methods of working. Adaptation and innovation in an industrial society present essentially an educational problem. A much clearer understanding of the problems of adaptation in an industrial society is needed and, as Dr. Taylor adds, a more

positive approach to the educational problems involved is overdue.

There is sense and insight in Dr. Taylor's comments on management, on the inherent weakness of controls and of organization, necessary as they are. If management must bear the major share of responsibility for restrictive practices, the fundamental need is leadership at all levels, and, in this, individuality and personality count to an extent that educational authorities—and others—are liable to forget. Moreover, the type of leadership we need to-day will not concentrate on economic efficiency to the detriment of the rights and dignities of the individual. However, here Dr. Taylor recognizes that national policy also has a part to play, particularly if we are to take work to the workers rather than transfer workers to the work.

While this group of lectures and addresses is of interest as illustrating the outlook and approach of responsible leaders within the chemical industry as much as for their diagnosis of some defects on which the National Economic Development Council has commented, the importance attached to management is impressive and supports much that was said at the recent Cambridge meeting of the British Association for the Advancement of Science. The idea that the chemical industry generally is backward is scarcely tenable in the light of these addresses, but their challenge to education and to Government is unmistakable. Neither in industry nor in Government is there room for complacency, and in the chemical industry as elsewhere the challenge of Britain's economic position will only be met if the response of efficient management and of workers and staff is matched by appropriate Government policy and structure for the assessments on which policy should be based.

NOBEL LECTURES AND SCIENTIFIC METHOD

Physics

Nobel Lectures including presentation speeches and Laureates' Biographies 1922–1941. Pp. xii+456. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 160*s*.

THIS handsome volume is the second of three volumes which present the Nobel Prize Lectures in Physics in the English language accompanied by the presentation addresses and short biographical notes. The Nobel Foundation hopes that they will serve to supplement its annual publication *Les Prix Nobel*. The publishers state that the translations of Lectures which were not given in English were prepared by the Babylon Translation Service of London: it is to be regretted that the English is often irritatingly bad, for example, "Allow me to refresh your memories what is the Laue phenomenon" (p. 254). It is curious, also, to have the short sentence which justifies the bestowal of the Prize listed, in each case, as "Motivation" in the contents. The Lectures are preceded by presentation addresses which are a valuable aid to the reader, although these sometimes exceed in length the Lectures by the laureates.

The period 1922–41 is a fascinating one, since it contains the development of revolutionary ideas associated with the names of Bohr, de Broglie, Heisenberg, Schrödinger and Dirac, as well as vital experiments concerned with the ultimate structure of matter made by laureates from Millikan (1923) to Fermi (1938) and Lawrence (1939). The Lectures are therefore particularly interesting from the point of view of scientific method and the philosophy of science, since they exemplify the modern method of starting with mathematical equations and then attempting to interpret their solutions later. This, of course, does not supply causal explanations but permits predictions which, it is nowadays claimed, are all that can be hoped for.

Prince Louis Victor de Broglie (laureate 1929) was the pioneer of the hypothesis of matter-waves. He says, "experiment which is the final judge of theories has shown that the phenomenon of electron diffraction by crystals actually exists and that it obeys exactly and quantitatively the laws of wave mechanics" (p. 255), and concludes "this wave is no myth". He nowhere actually states what the waves are waves of, and he accepts the prevailing word-magic which, by using the Latin word *quanta*, avoids saying what the quantities are quantities of. He appears to avoid actually calling these quantities 'energy'—rightly, because this is a purely metrical term which, therefore, could never be absorbed by any atom and cannot constitute a causal explanation. In recent years de Broglie has resumed the attempt he made in 1927 to give a causal interpretation to wave-mechanics; he has now, he says, "the growing impression that the majority of physicists who have yielded to these exaggerated abstract tendencies have too easily desisted from forming an intelligible representation of the phenomena of quanta physics"[1].

In 1932 the laureate was Werner Heisenberg—"for the creation of quantum mechanics . . .". He put forward the well-known thesis that we must forgo a visual description of the atom and maintained that the laws of Nature are basically statistical.

Erwin Schrödinger (1933) summed up his epistemological views somewhat obscurely as follows: "I would define the present state of our knowledge as follows. The ray or particle path corresponds to a *longitudinal* relationship of the propagation process (i.e., in the *direction* of propagation), the wave surface on the other hand to a *transversal* relationship (i.e., *normal* to it). *Both* relationships are without doubt real: one is proved by photographed particle paths, the other by interference experiments. To combine both in a uniform system has proved impossible so far. Only in extreme cases does either the transversal, shell-shaped or the radial, longitudinal relationship predominate to such an extent that we *think* we can make do with the wave theory alone or with the particle theory alone".

Paul A. M. Dirac, who shared the 1933 Prize with Schrödinger, explains how he derived a relativistic wave-equation involving the invention of new mathematical variables, and states that these "give rise to the spin of the electron" (p. 322), but he also admits frankly that these variables also "give rise" to some rather unexpected phenomena. (Mathematical terms and operators cannot, of course, "give rise" to anything physical. They can only give rise to other mathematical expressions. This illustrates the confusing misuse of language in which this subject is involved.) One unexpected phenomenon is that "an electron . . . must actually have a very high-frequency oscillatory motion of small amplitude superimposed on the regular motion which appears to us". As a result, "the velocity of the electron at any time equals the velocity of light" (p. 322): he does not comment on the fact that this contradicts the theory of relativity from which the conclusion has been derived, "but one must believe in this consequence of the theory, since consequences of the theory which are inseparably bound up with this one, such as the law of scattering of light by an electron, are confirmed by experiment". Scientists have not always agreed with this attitude to a theory: it raises the difficult problem of the status of an equation some of the interpretations of which are verified, and some are not, or are meaningless, or even contradictory. With equal frankness, Dirac discusses another difficulty—that the equations which led to the prediction of the positron also predicted negative energy-states, which are, of course, physically meaningless; but once again "we must

find some meaning for these states". In this case he is driven to the *ad hoc* hypothesis that they are nearly all "occupied" by electrons and to the further *ad hoc* hypothesis that if they were fully occupied they would be completely unobservable. Unoccupied negative energy-states are first called "holes" ("for brevity") and then a hole is said to be "just like an ordinary particle", and finally, the hole is called a positron. But despite the *ad hoc* hypotheses and the word-magic, energy-states are not particles, whether occupied or not, and are not observed but calculated.

In 1937, the laureates were Clinton J. Davisson and G. P. Thomson. In Thomson we see the spirit of British empiricism and a cautious and critical approach: "I am predisposed by nature in favour of the most mechanical explanation possible . . . it might be best, and it is certainly safer, to keep strictly to the facts and regard the wave-equation as merely a way of predicting the results of experiments".

After several unsuccessful attempts to give some physical meaning to matter-waves, mathematical physicists have realized that Eddington was right in calling wave mechanics "a dodge—and a very good dodge too"[2]. But dodges are not physical theories, and diffraction does not necessarily imply waves[3]. We still await a Nobel Lecture which will give us a physical theory of the atom.

G. Burniston Brown

[1] *The Current Interpretation of Wave Mechanics—A Critical Study*, preface (Elsevier, 1964).
[2] *The Nature of the Physical World*, 219 (Cambridge University Press, 1928).
[3] *Contemporary Physics*, **5**, 1, 15 (1963).

ELECTROCHEMISTRY

The Encyclopedia of Electrochemistry

Edited by Clifford A. Hampel. Pp. xviii + 1206. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1964.) 35 dollars.

Proceedings of the First Australian Conference on Electrochemistry

Held in Sydney, 13th–15th February, and Hobart, 18th–20th February, 1963. Edited by J. A. Friend and F. Gutmann, assisted by J. W. Hayes. Pp. xvi + 954. (London and New York: Pergamon Press, 1965.) 200*s*. net.

MANY of us will have our own, we suspect, limited ideas as to the scope of electrochemistry, and an 'encyclopaedia' in this field might accordingly appear to be a publishers 'gimmick' rather than a worth-while proposition to the potential user. An evening's perusal of the present volume, however, will convince even the most complacent reader that here is a book which not only fully justifies the title "*Encyclopedia*" but is also an authoritative up-to-date reference book of high scientific standard. The individual items, numbering 400–500 and written by nearly 300 special contributors, are substantial articles; they average about 2,000 words or more, are well supported by block diagrams, photographs and data tables and in addition in most cases conclude with relevant and important literature references. The items covered are theoretical and practical, laboratory and industrial, as indicated briefly by the following range quoted in the introduction: batteries, corrosion, insulation, electrodeposition, luminescence, electro-organic, electrothermics, industrial processes, theoretical electrochemistry and electrochemistry in biology and medicine. But such a classification does not convey anything of the wide range of substantial articles in each of these fields, nor prepare the reader for those no less important articles that cannot be so classified. Anyone whose work or interests impinge on matters in any way connected with electrochemistry in its widest sense is sure to find articles of direct interest in this book. The editor is by no means exaggerating when he states that this book will be especially useful to physical, inorganic and analytical chemists, to chemical corrosion, electronic and electroplating engineers, to metallurgists concerned with non-ferrous metals and with basic studies of high temperature and surface phenomena, to physicists specializing in electronics and to workers in biophysics and neurology.

To proceed from the general to the particular and discuss specific articles may not be helpful because of the wide range of readers catered for. The university student, for example, will be attracted by such articles as those by Dr. Parsons on the double-layer and on electrode kinetics, by Dr. Eisenberg on concentration polarization and by Prof. Read on hydrogen embrittlement. Nor will he be confused by electrode sign conventions; the European and American conventions are both clearly discussed and unambiguously used throughout with the American convention reserved for oxidation potentials. Thus those to whom the use of the American sign convention in text-books of electrochemistry is anathema may safely refer their student charges to this book. But to mention in this way items of particular interest to one class of reader is to single out just a few to the detriment of the hundreds of other items equally demanding recognition. One high-light which must be mentioned, however, is the inclusion of a most comprehensive "Table of Electrode Potentials and Temperature Coefficients".

This, then, is a book which, though it may have arrived unasked for and unexpectedly, nevertheless has arrived and is here to stay. The contributors are all acknowledged specialists in their fields and each has now confirmed or enhanced his own reputation. Not least in this respect is the editor, C. A. Hampel, who may feel justly proud of his success in harnessing so much electrochemical knowledge in one substantial volume. Typographical errors are insignificant; one might, however, wonder what is meant by "incomprehensible" liquid (p. 159), and one could be misled by the use of "free enthalpy" (p. 429) when referring to Gibbs free energy.

The justification for the First Australian Conference on Electrochemistry embracing virtually all the modern facets of electrochemistry, pure and applied, lies in two facts. The first is the present considerable interest in electrochemistry, reflected in a four-fold post-war increase in the percentage of physical chemistry research papers appearing in the field of electrochemistry; the second is the significant proportion of this work which is being carried out in Australia. The volume under review represents a substantial contribution to present-day electrochemical literature and offers unequivocal evidence of the success of this Conference. It is certainly a publication which should be read in part or in its entirety by all those interested in electrochemistry; our only reservation springs from the fact that *Proceedings* of conferences, even international ones such as this, often do not find their way into published abstracts. One wonders if the many original contributions would eventually reach a wider public had they been published in the usual journals and the present *Proceedings* limited to extended abstracts. This, however, is a fault of the system, not of the Conference.

The *Proceedings* contain, with the exception of two papers, a full and detailed record of all the contributions amounting to some 60 papers. About one-half of these were contributed by visiting scientists mainly from the United States and Canada, although several were from European, Japanese, Indian and Egyptian workers. Contributions by Russian workers were unfortunately absent.

The Conference was held partly in Sydney and partly in Hobart. Each of the twelve sections of the Conference was introduced by a different chairman; this provided the key to the success of the Conference and contributed in no small measure to the justification for this book. The choice of chairmen, all distinguished in their field, was excellent, and each in his address provided a lucid and detailed review

of the main theme and so prepared the way for the succeeding contributed papers. The range of topics covered is well illustrated by the following list of the twelve sections: solid-state chemistry, thermodynamics of electrolytes, corrosion, theory of double-layers, electro-analytical methods, applications (electroplating, anodizing), non-aqueous electrolytes, molten salts, fuel cells, electrode processes, electrochemical processes, electrowinning and electrorefining. The major sections are those concerned with fundamental research, and these give substance to Prof. J. O'M. Bockris's comments in the foreword that electrochemical research is now heavily concerned with electrode kinetics ('electrodics') and with kinetics and theories of electrode processes to the virtual exclusion of thermodynamic equilibrium studies. The sections on applied electrochemistry will prove no less interesting to those concerned with applications, but we consider that these sections might with advantage have been brought together, so permitting a greater continuity in the theoretical sections.

It is invidious perhaps to single out individual contributions. The review papers by chairmen Ross Macdonald (and C. A. Barlow) and by J. O'M. Bockris on the theory of the double-layer and electrode processes respectively are of particular interest because of the difficulties attending work in these fields. Perhaps not unexpectedly the number of contributions to these sections were not as numerous as in some of the other sections. In the section on electrode processes, a paper by Horiuti, Matsuda, Enyo and Kita on the mechanism of the hydrogen evolution reaction is of considerable interest, as also is that by Christov on the application of quantum mechanics to electrode kinetics. In the section on electrochemical processes mention should be made of the chairman's address and a stimulating paper by Bockris, Devanathan and Müller on the structure of charged interfaces. But this is to mention a few among the many excellent papers the degree of importance of which will vary according to the particular interests of the reader.

The production of the book is excellent, and Pergamon Press have fully maintained the high standard set by F. Gutmann and M. Bloom, who organized the Conference and, with J. A. Friend, edited the *Proceedings*. The price at £10 is not excessive for such a book.

R. F. Phillips
N. A. Hampson

NUCLEAR AND RADIOCHEMISTRY

Nuclear and Radiochemistry

By Dr. Gerhart Friedlander, Joseph W. Kennedy and Dr. Julian Malcolm Miller. Second edition. Pp. xi + 585. (New York and London: John Wiley and Sons, Inc., 1964.) 68*s*.

"FRIEDLANDER, Kennedy and Miller" is still recognizably the same excellent text as "Friedlander and Kennedy", though the format is new, the order of the chapters has been changed, and there are substantial additions to the subject-matter, as well as some deletions. On balance, the new edition is longer by roughly one-third compared with its predecessor. Prof. J. M. Miller of Columbia University has replaced the late Prof. J. W. Kennedy as author, though Kennedy's name has deservedly been retained on the title-page. Not unnaturally, the book still has an American slant, but this in no way invalidates it for other countries.

In their preface the authors state that "the book is still intended as a text for an advanced undergraduate or first-year graduate course; but a significant aspect of its modernization is found in the greater depth of treatment accorded to many topics, particularly theoretical ones". The new material includes greatly expanded treatments of nuclear reactions and radioactive decay processes, accounts of new types of accelerators, counters and other instruments, and two completely new chapters, the first on models of the nucleus, and the second on the Mössbauer effect and other nuclear processes that can be made to yield chemical information.

It is characteristic of the book that, whatever topic one turns to, one finds the essential points well and clearly stated. It has been my experience more than once, after wrestling with a tricky point, to find the conclusion that I have extracted with difficulty from other sources set down in simple terms in *Nuclear and Radiochemistry*.

The title and contents of the book prompt the questions: What is nuclear chemistry? and What is radiochemistry? The claim that the book is "written by chemists for chemistry students" is a valid one, yet of the fifteen chapters, only three deal with specifically chemical topics, and only two others contain sections of any length on chemical subjects. Moreover, the chapter on radioactive tracers has actually been shortened in the new edition.

I first remember the term 'nuclear chemistry' in the annual reports of the Chemical Society for 1935, when it was used to refer to the science of nuclear transmutations, on the analogy between chemical reactions, in which atoms are conserved, and nuclear reactions, in which nucleons are conserved. It is now often used to mean something like "nuclear studies carried out by chemists". Some of the properties investigated in this context undoubtedly belong to the realm of physics rather than chemistry, but chemists do a large share of the work because success depends on preparing pure specimens and on carrying out efficient chemical separations at the end of the experiment.

However, the term is also used in a much wider sense. Coryell, in his foreword to the English edition of Haissinsky's *Nuclear Chemistry and its Applications*, speaks of it including "nuclear reactions, radioelements, radiochemistry, isotope chemistry, radiation chemistry, and tracer applications", and all those fields and more are indeed covered in Haissinsky's book, even though Haissinsky's own definition of nuclear chemistry is merely "the study of the transmutations and transformations of nuclei".

'Radiochemistry' is likewise used in both broad and restricted senses. Originally it referred to the chemical effects of ionizing radiations, but this is now a matter of history only. Later it was applied to the chemistry associated with the natural radioelements, though excluding the ordinary chemistry of thorium and uranium, in which radioactivity played no part. When artificial radioactivity was discovered, the term was of course extended to the new radioactive species. A further extension was to the radiotracer field, in which both natural and artificial radioelements find numerous applications. Many authors still stop at this point, and leave the lively subjects of hot atom chemistry and nuclear chemistry (in the limited sense) largely outside the scope of 'radiochemistry'. Others again treat the subject as if it were primarily a collection of manipulatory and measuring techniques. *Radiochimica Acta*, on the other hand, adopts a more eclectic view—one which I also favour. There seems indeed no good reason why 'radiochemistry' should not include all aspects of the interaction of chemistry with radioactivity, with the sole exception of most of the radiation chemistry aspects, which involve by and large a distinct set of concepts and techniques.

This discussion is relevant to the book under review, because it needs to be appreciated that *Nuclear and Radiochemistry* is primarily a text-book of nuclear chemistry in the restricted sense, and only secondarily a general text-book of radiochemistry. The nucleus itself, nuclear reactions and radioactivity are discussed very fully, since they form the principal subject of investigation in nuclear chemistry, and so are the techniques needed. Other radiochemical topics are treated much more briefly, and very largely from the point of view of the nuclear chemist. In saying this, no criticism is intended, but rather a definition of the scope of this important work. H. A. C. McKay

FOURIER THEORY

A Treatise on Trigonometric Series

By N. K. Bary. Vol. 1: Pp. xxiii + 553. 84*s*. net. Vol. 2: Pp. xix + 508. 105*s*. net. (London and New York: Pergamon Press, 1964.)

PROF. Nina Bary, distinguished for her contributions to the theory of functions of a real variable, died in 1961, the year of publication of her book on trigonometric series of which this is a translation. Zygmund's much-extended two-volume revised edition of his book on the same topic appeared in 1959, when Prof. Bary's was already in proof, so that we now have two independent and up-to-date treatises, each with much the same aim: to lay firm foundations of the theory and to carry the work, in suitably selected regions, to the frontiers of present knowledge. Both books are intended for the pure mathematician who can claim some acquaintance with set theory and the Lebesgue integral; there is nothing for the applied mathematician or physicist. The total amount of material is about the same in the two books; but Zygmund deals with several topics which Bary does not touch, while she has only two chapters not covered in Zygmund. The difference here is due to the slower pace of the Russian book; Zygmund demands throughout a fairly high level of mathematical sophistication, whereas Bary takes things at a more leisurely pace, particularly in her first volume. The phrase "it is easily seen" has been used, often in a Pickwickian sense, by many authors since Laplace set a bad example; in her preface, Bary protests against its frequent misuse, and has endeavoured to make her text, at least in the first volume, easily readable by the good undergraduate. To some of the longer and more delicate proofs, she appends informal comment to sketch the main lines and to emphasize the critical points of the argument.

After some preparatory analytical matters, a long first chapter of some 170 pages gives a good general survey of the domain, including the Dini and Jordan tests for convergence, the Gibbs phenomenon, Cesàro and Abel summability, Poisson's integral. Chapter 2 deals with the order of magnitude of Fourier coefficients of certain types of function, and Parseval's theorem. The next three chapters take the natural line of examining the convergence of a Fourier series at a point, the uniform convergence of Fourier series of continuous functions, and convergence in a point-set. Most of this is to be found in the first half of Zygmund's first volume, but the last chapter deals with work by Men'shov on a problem which Zygmund does not touch: roughly, given a function $f(x)$, can it be altered on a set of arbitrarily small measure to give an 'adjusted' function the Fourier series of which will converge everywhere or almost everywhere, or converge uniformly ?

Vol. 2 begins with summability processes, the simpler part of the theory of the conjugate series, absolute convergence, sine and cosine series with coefficents which tend to zero, and the remarkable differences which arise when the series is lacunary, having increasingly large gaps. Then there are chapters on the convergence, absolute convergence, and uniqueness of the general trigonometric series: in the main, these topics centre around what can be said about a point set on which the series has a certain specified behaviour. The final chapter deals with another problem of Men'shov's, again not discussed by Zygmund: this concerns the representation of a function by a summable trigonometric series, arising from work by Lusin and Privalov which showed that if a function is finite almost everywhere (in the basic 2π interval) then a trigonometric series can be found which is summable to the function by the Riemann, Poisson or Cesàro methods.

The techniques used are on the whole not so advanced as those which Zygmund draws on, so that a good deal of his second volume lies outside the range of Bary's book. The student thinking about research in this field might well begin and go a long way with Bary, turning to Zygmund for the sharper weapons likely to produce a deeper penetration.

There are some infelicities of expression, possibly due to the need to keep the translation close to the original. In some places, it is said that an assertion "cannot" be true, when what is meant is that it "need not" be true: the distinction is important, and blurring it may confuse the novice. But in general Mrs. Mullins has coped well with a gigantic task.

T. A. A. Broadbent

ERGONOMICS

Ergonomics

Man in his Working Environment. By K. F. H. Murrell. Pp. xiv + 396. (London: Chapman and Hall, Ltd., 1965.) 63*s*.

ERGONOMICS: Man in his Working Environment provides an important link between scientific research and industry. It is a text-book both for investigators in the basic sciences concerned with the practical application of their research, and for industrialists and designers who appreciate the importance of the human factor in maximizing industrial efficiency.

Ergonomics has been defined as "the scientific study of the relationship between man and his working environment", but Mr. Murrell has extended the term 'environment'. The key *motif* of ergonomics is that of fitting the work situations as a whole to the man on the basis of scientific knowledge, including knowledge of human physiological and psychological capacities. This is called 'human engineering' in the United States. It is an interdisciplinary applied science relating principles derived from the basic sciences to work design, the control of physical environment factors and the mode of work organization.

With more and more semi-automatic and automatic processes being introduced into industry, the contention is that when equipment is intended for human use, it should, so far as possible, be designed to give effect in a man–machine unit, with the man complementary to the machine and the machine complementary to the abilities of the man. While physical stress has tended to be reduced in modern industry, the shift of emphasis from man as 'doers' to man as 'controllers' has tended to increase psychological stress. Stress can lead to inefficiency and reduced output, through failure to match the requirements of the task with the capacity of the operator. Again, the contribution of ergonomics to work design is invaluable when technical change makes it necessary for operators to move on to jobs which may be variants of their old jobs or entirely new.

As a dual text-book for scientists and industrialists the book is well designed. Part 1 outlines salient data from physiology, anatomy, psychology, etc., while Part 2 deals with practical ergonomics and gives examples of the application of ergonomic principles in work design and manpower utilization. The book is easy reading; technical and scientific jargon having been largely avoided. Mr. Murrell points out that while there is a large body of data in scientific publications which is relevant to industry, there is need for some translation to put this to work. He contributes materially to such a translation, and this publication contains an extensive reference section to his material.

The chapter on the design of seating provides an example of the universality of ergonomic contribution to human well-being. The importance of good seating design is becoming more widely recognized. While considerable research has been done in seating design, surprisingly little of that research has been put into practical application. This chapter sets out the requirements of good seating as ascertained from research, summarizes the relevant anthropometric data and gives practical advice for seating-manufacturers.

Significantly, Mr. Murrell's final chapter deals with the necessity of taking account of ageing in the interests of ensuring the maximum efficiency of available manpower. The importance of this is underlined by the fact that more than 50 per cent of the male workpower in British manufacturing industry is already over the age of forty. In certain circumstances efficiency can be affected by the concomitants of ageing from as early as thirty-five. Yet industry has shown little interest in research on ageing in relation to occupation. In our present demographic circumstances there is need to retain or engage older workers. But industrial gerontological research emphasizes the basic necessity of appropriate work-changes, even from the middle-age, if older manpower is to be used to the best effect. The author relates age-indicated work-change to that resulting from technical developments. In both, the use of ergonomic principles would result in a better matching of job demands and physiological and psychological capacities. This may be either through a shift to an alternative job, that is change 'of' the job, or some modification of the work demands, that is change 'in' the job. The latter alternative gives greater opportunity for the continued use of the previously acquired skills and experience.

It is evident that ergonomics can make an important contribution to the more efficient and more considerate use of manpower. This book will also be of interest to non-specialists who wish to be conversant with the relationship between science and its practical application in the modern world.

C. E. Fleming

REGULATION OF GROWTH

Adaptive Growth

By Prof. Richard J. Goss. Pp. 360. (London: Logos Press, Ltd., in association with Elek Books, Ltd. Distributed by Academic Press, London and New York, 1964.) 70*s*.

THIS book will be welcomed by all biologists and pathologists interested in vertebrate development, for it fills a gap in the bibliography of this subject. Another attraction is that it is written by a single author, who has something definite to say and who says it well.

Prof. Goss has set out to explain diverse kinds of post-embryonic growth in terms of a unifying hypothesis, namely that the regulation of growth is governed by the functional demands of the organism. He is fully aware of the teleological overtones of the word 'demand' and carefully avoids them in his attempt to elucidate the mechanisms which control the homoeostasis of growth. The characteristics of different types of developmental phenomena, such as the local control of wound healing, or the systemic mediation of compensatory growth, are interpreted as adaptive responses determined by the function of the organ concerned and the nature of the stimulus. Normal growth is discussed in terms of cell turnover, different modes of proliferation of cell populations are classified, and ageing—"the body's homoeostatic depreciation"—in some tissues is briefly reviewed.

Nearly half the chapters of this book are devoted to an analysis of compensatory growth which is exhaustively considered on gross, histological, cellular and molecular levels. Evidence of the action of humoral activators and inhibitors is critically examined. The liver and haematopoietic system predominate in this analysis, as the majority of investigations have been concerned with these tissues, but all other organs which show compensatory growth are dealt with. The adaptive role of the pituitary-thyroid system could have been more fully described, particularly the functional significance of the relay system.

No attempt has been made to review growth in all vertebrate groups. No data on fishes or reptiles are included, and little on birds. Perhaps this is because little space has been devoted to morphogenetic regeneration (apart from an interesting chapter on amphibian lens regeneration) although the author himself has made outstanding contributions to this subject. He does, however, briefly mention Singer's and Thornton's investigations, commenting on the significance of the role of nerves in limb morphogenesis.

The chief task of this monograph is to re-define all growth phenomena according to functional ideas. As the author points out in his preface: "It has become fashionable to disregard functional considerations in favour of mechanisms by which the regulation of tissue mass might be an end in itself. Consequently, much of to-day's research is predicated on the assumption that humoral agents are responsible for adjusting the sizes of organs to certain predetermined dimensions".

While the author does not deny the participation of humoral agents in the control of mitosis of many tissues, he postulates that they are primarily dependent on the physiological demands of the particular tissue. Thus the stimulation of erythropoiesis is mediated through the same regulatory mechanisms whether the initial functional insufficiency was due to loss of blood or reduced oxygen in the environment. Likewise the mechanisms controlling epidermal hyperplasia are the same in wound-healing and the growth of calluses. This interpretation is controversial. It is not the view, for example, of Prof. Bullough, whose ingenious experiments on mouse epidermis and whose theory of mitotic inhibition by chalones are reviewed by Goss.

Moreover, there seems to me to be a deeper contradiction between a 'chalone' theory of mitotic regulation and the functional demand theory. Prof. Swann, a proponent of the functional demand theory himself, has pointed out that mitosis is a form of differentiation in requiring specialized (but not tissue-specific) protein synthesis which must therefore be antagonistic to tissue-specific synthesis, and Glinos has suggested that functional overload of tissue-specific synthesis (in the liver) switches the synthetic machinery of the cell to elaborate mitotic proteins. The chalone theory recognizes the antagonism, but reverses the action of the products of differentiation (chalones) so that they inhibit mitoses, or, as recently stated in more contemporary terminology, the concentration of a chalone has an effector-like action "which determines whether the 'mitosis-operon' or the 'tissue-operon' is active"[1]. In renewing cell systems, such as epidermis or blood cells, a single stimulus could derepress the synthesis of both mitotic proteins and tissue proteins, since proliferation and differentiation represent separated compartments of the cells' life-histories. In expanding cell systems, however, where there is no distinction between stem-cells and functional tissue (for example, liver), partial ablation will cause a substantial rise in the mitotic index of the remaining tissue just at the time when the functional demands for tissue-specific products are greatest (though it is not yet certain whether an increase in the tissue-specific protein synthesis per cell occurs simultaneously). While Prof. Goss reviews in some detail the various theories of mitotic control and the data supporting them, he does not clearly define this conflict between immediate and long-term homoeostasis of functional activity, which must occur if mitosis and differentiation are competitive or mutually exclusive. If products of differentiated tissues normally inhibit mitosis and further tissue-specific synthesis, then a fall in their concentration following partial ablation will promote both activities, though it remains to be explained how different cells in an expanding system are allocated to the alternative pathways.

Since this monograph represents a virtuoso performance over a wide repertory, it is not surprising to find occasional factual inaccuracies and interpretative inconsistencies. Thus on p. 88, Prof. Goss is of the opinion that "cell migration is antagonistic to cell division", stating that "Abercrombie and Ambrose suggest that until the motile cells in a healing wound again become station-

ary as a result of contact inhibition, their mitotic propensities are temporarily suspended". In fact they suggest the opposite, which Prof. Goss himself corroborates five pages later when discussing Harding and Srinivasan's observations on small wounds in lens epidermis.

Prof. Goss includes a chapter on maternal–foetal relationships in which he reviews the poorly understood interactions between homologous organs in this "special case of parabiosis". There is also an interesting chapter on allometry in which the author discusses the functional, histological adaptations in some organs relating to the size of different species, and the adjustments in size which may occur following transplantation of organs. In a delightful account of the allometric relationships of urodeles varying in ploidy, we see that although cell size varies according to the number of chromosome sets, the size of gross and histological structures is constant, indicating that it does not matter how the chromosomes are packaged, their total number being the controlling factor. This can lead to certain developmental dilemmas, such as when the size of an 'organ' approximates to the size of the diploid cell. Triploid erythrocytes can pass through capillaries of normal (diploid) diameter (by change in shape, which gives a clue as to how their shape is controlled), but the single pair of Mauthner's cells cannot remain proportional both to ploidy and brain size.

Prof. Goss mentions Fankhauser's discovery that triploid salamanders are less intelligent than diploids, and he states that "the quality of the central nervous system cannot be improved by increasing the size of neurones at the expense of their numbers". It is disappointing that Goss does not actually pose questions about the lack of compensatory growth of the brain, though he does incidentally provide most of the answers! There are several unsolved problems concerning the different sizes of neurones. Whereas hypertrophy of particular nerve cells occurs, usually as a consequence of functional activity, neuronal hyperplasia is not known. Goss prefers to regard mitotic incapability as an adaptation serving to prevent disruption of organized cell communities rather than an unfortunate consequence of specialized differentiation. It is the function of the cell, not its "degree of specialization", that governs its ability to divide. Neither is it functional in the case of nerves and muscles to maintain 'mitotic competence' by recruitment from undifferentiated stem-cells.

Prof. Goss brings a fresh and firmly biological approach to his analysis of regulatory phenomena in growth. His controversial ideas should lead to lively discussions and penetrating research during the next few years.

R. A. Weiss

[1] Bullough, W. S., and Rytömaa, T., *Nature*, **205**, 573 (1965).

MICROLEPIDOPTERA UNDER THE MICROSCOPE

Catalogue of the Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick

By J. F. Gates Clarke. Vol. 5. Pp. 581 (283 plates). (London: British Museum (Natural History), 1965.) 300*s*.

BIOLOGISTS do not often realize the immensity of the task still to be undertaken and the vast amount of published work needed before even half the species of the world can be named even by specialists. Much of the work was (and a considerable amount still is) done by expert amateur entomologists. Meyrick's position as an amateur entomologist was perhaps unique because of the extent of his work. Although well up to the standards of his time, his failure to illustrate his new species created a world problem. He first published descriptions of new species of microlepidoptera in 1886 when he was a schoolmaster in New Zealand, and he gradually came to dominate their study after his return to England. He retired in 1914 after many years teaching classics at Marlborough College. Specimens of microlepidoptera were sent to him by collectors and museums from all parts of the world, but he retained in his collection, which is now in London, only about a third of the types. By the time of his death in 1938 he had published descriptions of about 15,000 new species (catalogued in Volume 1).

Dr. Gates Clarke became an authority on the microlepidoptera through his work for the United States Department of Agriculture and the Smithsonian Institution, and found some years ago that further taxonomic progress was impossible until Meyrick's species had been re-examined. He then worked for some years at the British Museum (Natural History) and the Trustees gave him permission to dissect Meyrick's types. The abdomens of the 5,000 moths were carefully removed, softened, dissected and mounted on microscope slides. This has for the most part been carried out with commendable skill, but it would be interesting to know whether the large numbers of Cosmopterygid male genitalia were mounted sideways from choice, or whether their symmetrical arrangement presented peculiar difficulties.

Volumes 1 and 2 of this work were published as long ago as 1955 and it is very much hoped that the final one or two volumes will soon be completed. This, the fifth volume, dealing with moths of the families Timyridae, Hyponomeutidae, Ethmidae, Metachandidae, Cosmopterygidae, Walshiidae, Blastodacnidae and Scythridae, contains 283 half-tone plates some of which include as many as 15 illustrations. For each species there is a photograph of the wings of one side of the type specimen and sometimes a picture (which gives rather little detailed information) of the head and its associated sense organs. If the species is the type of a genus there is also a clear drawing of the wing venation. Photomicrographs of dissections of the genitalia of the types provide a much-needed supplement to Meyrick's original description. The quality of the photographic illustrations is almost uniformly good, so that in spite of difficulties with depth of focus they are almost as useful as good drawings would be.

Apart from a very few instances where the author synonymizes two species, the text consists of little more than a legend for the illustrations and the reference to the original description of the species concerned. Lectotypes are designated when needed and Meyrick's occasional errors in determining the sex of his specimens are corrected.

When this series of volumes is complete, the stage will be set for the next advance. This will be the revision of genera, including species described by many authors. That there is much to do is clear even from a superficial study of the diversity of form in such genera as *Lecithocera* and *Ethmia*. Dr. Gates Clarke has wisely not tried to do two things at once, for the present work is already large and expensive. The speed with which these much-needed revisions come will depend very largely on the funds available for this basic kind of work in museums and universities. The Trustees of the British Museum (Natural History) already publish a very substantial amount, but publications like this are, unfortunately, still far too rare. Of the hundreds of thousands of type specimens in the museums of the world a surprisingly high proportion must still await critical re-examination. Experience suggests that the original descriptions have been misunderstood in at least one-tenth of the cases. Many of the changes in the names of animals which so baffle the non-specialist are caused by the correction of these old mistakes of identification.

Dr. Gates Clarke is to be congratulated on performing this basic and useful task, and the staff at the British Museum (Natural History), especially Mr. J. D. Bradley, who has assisted in the work at every stage, will earn the thanks of the many specialists in various parts of the world who will find this work indispensable.

G. C. Varley

Man's Place in the Island Ecosystem

A Symposium. Edited by F. R. Fosberg. Pp. vii + 264. (Honolulu, Hawaii: Bishop Museum Press, 1963.) 8 dollars.

WHEN the late Sir Arthur Tansley in 1935 introduced the term ecosystem and later defined it as "a unit of vegetation considered as a system . . . includes not only the plants but also the animals habitually associated with them", it may be doubted whether he envisaged man as the most important animal concerned. Yet this is the logical conclusion, and where better to study the problems involved than among the numerous and varied islands of the vast Pacific Ocean? An island is a clearly defined areal unit; in the Pacific there is every stage from the uninhabited coral islet to the crowded sophisticated human atmosphere of the United States' fiftieth State. Under the Humid Tropics programme of Unesco, a symposium was arranged to take place at the tenth Pacific Science Congress held in the University of Hawaii at Honolulu in August 1961. It was very efficiently organized by Dr. F. R. Fosberg (Washington), aided by his able lieutenant, Dr. Marie-Hélène Sachet, and he contributes the short introductory paper on the ecosystem concept.

The major criticism must be that too much was attempted in the time allotted. Each of the thirteen papers is of the nature of an *ex cathedra* statement and each was followed by a comment by a 'discussant'—limited to 3 or 5 min, despite the fact that he might have been flown half-way around the world to take part. It was soon clear that no time would be available for discussion; in a few cases a question or two after the discussant had spoken. Many of the big names in the ecological field from all over the world are there—Marston Bates (Michigan), Pierre Gourou (Brussels), G. Mangenot (Paris), Irene Taeuber (Princeton), William L. Thomas (California), Kenneth Cumberland (New Zealand)—among the contributors; J. A. Steers, H. E. Maude among discussants; while a distinguished audience is revealed by simple questions from Paul Sears and C. F. A. Pantin. The task of a general summing up was left to Oskar Spate (Canberra).

The symposium is a landmark in the present development of thought. The ecosystem was the concept of a botanist mindful of the interaction of plants and animals and of the significance of the complex environment with its various physical factors. Here the influence of man brings in the physical and human geographer with the concept of human ecology fitting into the whole. The time to absorb the several contributions was lacking in Honolulu; this comprehensive report allows time for the proper and careful study of a series of papers of the highest value and significance. The sequence is logical—from physical environment and marine influence to the nature and evolution of the biota, the reciprocal man–nature relationship, the pattern and varied nature of human influence and culture systems to the practical issues of the present problems of human pressure and demographic instabilities. This is an essential volume for every serious library. L. Dudley Stamp (Discussant)

A History of Parasitology

By W. D. Foster. Pp. vi + 202. (London and Edinburgh: E. and S. Livingstone Ltd., 1965.) 35*s*.

THIS is a well-written and interesting book and I gained much pleasure and information from it. It is the first account of the history of parasitology written in English, and a book that has long been needed.

The author provides, in his first chapter, a succinct account of the growth of parasitology from ancient times. In succeeding chapters, in order to illustrate the basic discoveries of parasitology, he outlines the history of our knowledge of a series of animals parasitic in man.

He begins with the trematodes, and discusses the liver fluke, *Fasciola hepatica*, and the schistosomes, which cause bilharziasis, which is still one of the major diseases of man and at present the subject of a campaign organized by the World Health Organization. Four nematode types are then discussed, namely the hookworms, *Trichinella spiralis*, the Medina worm (*Dracunculus medinensis*), and *Wuchereria bancrofti* (a cause of filariasis). He next considers, in a separate chapter, the parasitic Protozoa. In the chapters that follow he treats the trypanosomes, *Entamoeba histolytica*, the cause of amoebic dysentery, and those other Protozoa, the babesias, which cause serious diseases of cattle and other farm stock. A final chapter brings the history up to about the year 1920, when parasitology became well established as a branch of biology.

This plan has certain advantages, but it does tend to direct attention to the animals parasitic in man only, and some readers will wish that more attention had been given to the achievements of veterinary parasitologists. It is true that their work on the babesias is discussed, but more space might have been given to the part played in the development of parasitology by co-operation between the veterinarians and workers concerned with diseases of man. Moreover, a chapter on the social and economic effects of parasitic animals and the devastating effects of some of them on the food supplies of the world would not have been out of place, for there is much evidence that animals parasitic in man have, through their social and economic effects, influenced appreciably the course of human history. Diseases caused by them have, for example, greatly influenced, if they have not actually determined, the outcome of wars. But themes such as these were no doubt outside the author's intention, which was, he explains in his preface, "to give an accurate account of the basic discoveries of parasitology . . . at a level comprehensible to the non-professional parasitologist". In this general aim he has certainly succeeded. The book will be read with pleasure and profit by all who are interested in the subject. G. Lapage

Histologie und Mikroskopische Anatomie des Menschen

Von Dr. W. Bargmann. 5. überarbeitete Auflage. Pp. xvi + 856. (Stuttgart: Georg Thieme Verlag, 1964.) 72 D.M.

THE fact that Dr. Bargmann's book is, after fifteen years, already in its fifth German edition, and that the second Spanish edition is in preparation, speaks for the author's thoroughness in revision and his desire to keep up with recent developments. The text is based on a correlation of histology with physiology, biochemistry and submicroscopic morphology on one hand, and where applicable to pathology on the other. The text is divided into two parts. The first deals with general cytology and a descriptive exposé of the tissues: epithelial, supporting, muscular and nervous.

The second part considers the organs and organic systems: locomotor, circulatory and defensive, internal, secretory, digestive, respiratory, urinary, sexual, cutaneous, sensory, and central nervous systems. The text is lucid and integration of the different disciplines is excellent although some might find a more extensive discussion of tissue culture morphology of advantage. Literature references are ample and not confined to German publications. The illustrations are of excellent quality, but, as they are not indicated in the index, are sometimes hard to find if a particular one is sought. Another criticism, which applies to most publications, is that magnifications mentioned do not indicate whether they apply to the finished product or are applicable to some previous stage in their production. Both reference and subject indexes are adequate with the aforementioned reservation.

In conclusion this book is of general interest and, because of the illustrations, might even prove useful to workers who have only a limited knowledge of German.

G. Sander

Theory of Crystal Dislocations

By Prof. A. H. Cottrell. (Documents on Modern Physics.) Pp. ix+94. (London and Glasgow: Blackie and Son, Ltd., 1964.) 32*s*. 6*d*.

THIS little book (also available in paper-back form) is a kind of vade-mecum for dislocationists. It deals with the basic theory of dislocations, and consists mainly of the notes prepared for Prof. Cottrell's lectures to the 1956 summer school of the University of Grenoble at Les Houches. Some measure of the extent to which this theory has now been incorporated into the accepted physical scheme is provided by the fact that only minor changes were necessary when the manuscript was prepared for publication six years later.

It is perhaps unnecessary to add that this rather satisfactory situation does not extend to the complex many-dislocation problems which are encountered in plastic deformation. Developments in the theory of deformation, and in the experimental observation of dislocations by high-resolution techniques, are mentioned briefly in a series of very short notes added to the original manuscript. Although these additions were completed as recently as 1962, they have inevitably dated much more rapidly than the basic theory; the 'jog theory' of work-hardening, for example, is no longer current.

The book is written with Prof. Cottrell's usual clarity, but it should be emphasized that it is not a text-book from which to approach the basic theory for the first time. Its main use will undoubtedly be as a desk-book for quick ready reference, or as a companion of such slender dimensions as not to deter the aeroplane traveller. It is a great convenience to have so many useful results gathered together in compact form, and it may seem ungrateful to ask for more. However, there have been important developments in basic theory in the past few years, dealing, for example, with the stress fields of various types of dislocation aggregates, or with the theory of continuous distributions of dislocations. If Prof. Cottrell could find time to add these to a future edition, the value of the book would be even greater.

J. W. Christian

Molecular Orbital Theory

By C. J. Ballhausen and Dr. Harry B. Gray. (Frontiers in Chemistry.) Pp. ix+273. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 9.90 dollars.

THIS book is divided into two approximately equal parts, the first being a text of atomic orbitals using the LCAO approach, while the second part is a collection of previously published papers on the subject. A very simple and short exposition is given of the basic concepts of the LCAO theory, which leads into a group theoretical section on the uses to which these simple concepts may be put. These include symmetry consideration electronic states and hybridization. The types of possible bonding systems are discussed and the results applied to selected diatomic, triatomic and a few of the more simple polyatomic molecules. Finally, the theories developed are applied to more complicated polyatomic molecules making use of ligand field theory. The book is written in a simple manner with the result that the reader is sometimes left with the impression that the stage of knowledge of molecular theory is more advanced than is the case in reality. The illustrations are not only well done but are also so positioned in the text that they are extremely useful to those readers who are not very conversant with the more mathematical form of presentation. The choice of papers in the second part is in some cases a little surprising, but some very useful information has been collected together, making the book valuable as a reference for the reader interested in making molecular calculations.

E. W. T. Richards

The Evolution of the Bristol Channel

By F. J. North. Third edition, revised and enlarged. Pp. vii+110+14 plates. (Cardiff: National Museum of Wales, 1964.) 15*s*.

OVER a period of many years Dr. North has done much to interest the layman in geology, particularly in South Wales. In the third edition of *The Evolution of the Bristol Channel* one of his books is once more brought up to date, incorporating much recent work in this area. A historical introduction precedes a simple account of the geological processes of erosion, transport and sedimentation, and earth movements; and this is followed by a concise geological history of the region, with particular reference to the Welsh coast. Useful palaeogeographical maps and a geological time scale (the latter unfortunately not brought up to date) accompany accounts of the structure of the Welsh coalfield and of the geological reasons for the distribution of the mines. Similarly a description is given of how the formations exposed on the coast of South Wales control the form of the coast-line. The origin of the South Wales drainage pattern, the evolution of the Bristol Channel since its appearance in Pliocene times, the Recent changes in sea-level, the appearance of man, the accretion of sand dunes, and the great flood of the Wentloog Level in 1607 are among the many topics dealt with in an interesting manner. The plates and diagrams usefully supplement the text, though the standard of reproduction is not high. Visitors to the South Wales coast and the local inhabitants are fortunate in having available this very readable account of the local geological history.

A. R. MacGregor

Tizard

By Ronald W. Clark. Pp. xvii+458+16 plates. (London: Methuen and Co., Ltd., 1965.) 50*s*. net.

SIR HENRY TIZARD is still freshly remembered by many who worked with him or were associated with him in one or more of his varied activities. In the relatively short time since his death numerous tributes have been paid to his work, notably by Prof. P. M. S. Blackett and by Prof. R. V. Jones in Tizard Memorial Lectures, in Sir Harold Hartley's memoir for the Chemical Society, and by Sir W. S. Farren and Prof. R. V. Jones for the Royal Society. Nevertheless, there is a place for Mr. R. W. Clark's book, in which he has made full use of his access to unusually large collections of papers.

With one reservation, Mr. Clark has given us a well-balanced account of Tizard's life and work which does full justice to Tizard's real achievements. The reservation relates to the chapter on Tizard's post-war years as chairman of the Advisory Council on Scientific Policy and of the Defence Research Policy Committee, which scarcely conveys the significance of his positive achievements or the magnitude of his own contribution to the complex problems of the relations between science and Government. Mr. Clark, moreover, has avoided being drawn into personalities of the argument that has stemmed from Sir C. P. (now Lord) Snow's lecture on that theme, and his book points to most of Sir Henry's activities in the field of education. Its value is enhanced by a careful bibliography and references to most of Sir Henry's published papers. There is an error on p. 369, when a long letter to Sir Alan Barlow is attributed to Sir Henry himself instead of Sir Thomas Morton—an error which has since been rectified by Mr. Clark, who notes that the views expressed in it are substantially those of Sir Henry himself.

If the book is one to be studied by all who are concerned with the intricate problems of science and Government, it can scarcely be put down by a British reader without some sense of shame for the way in which Sir Henry was treated by the British Government. The exploitation of the scientific adviser moving in and out of the Civil Service is a reproach which we should be at pains to avoid in any structure established for civil science.

RECENT DEVELOPMENTS IN COSMOLOGY*

By Prof. F. HOYLE, F.R.S.
Professor of Astronomy and Experimental Philosophy, University of Cambridge

I SHALL start from the observed shift of the spectrum lines of galaxies interpreted in terms of the expansion of the universe. The red shift implies that distances between galaxies, measured with an imaginary ruler for example, increase with time. An immediate question is whether the universe was denser in the past than it is to-day. If so, how much denser was the universe ?

A definitive answer to this critical problem could be obtained in principle by observing the state of the universe in the past. This is possible because of the finite speed of light. We do not observe a galaxy as it is now but as it was at the moment the light started on its journey. In the case of very distant galaxies the light started several billion years ago, so we have direct evidence of the state of affairs several billion years ago. All that need be done, again in principle, is to observe the density of galaxies as it was a few billion years ago and to compare it with the density in our immediate neighbourhood. This would be a direct way of settling the density problem. This indeed was the way in which Hubble tried to settle it more than thirty years ago. He failed to do so because of the extreme difficulty of making a fair count of distant galaxies. The tendency is to count the brighter galaxies but to miss the fainter ones. Since Hubble's attempt nobody has had the hardihood to make a direct assault on the problem by attempting to count galaxies.

Ryle and his associates have counted radio sources instead of galaxies, and here the result has turned out to be much more clear-cut, at any rate so far as the counting process itself is concerned. The difficulty with radio sources is that we are still far from sure exactly what it is that is being counted. Developments in the past two years, the discovery of the quasi-stellar sources in particular, have shown the situation to be more complex than it was at first thought to be. The indication of the radio counts is that the universe was more dense in the past than it is to-day. However, further knowledge is needed concerning the nature of the radio sources before this conclusion can be regarded as definitive.

If the quasi-stellar objects are truly cosmological a great deal becomes pretty well settled. Imagine an object of fixed intrinsic brightness to be moved to increasingly great distances. Two things will happen. The apparent luminosity of the object will decrease and the red-shift of the spectrum lines will increase. A theoretical relation between these quantities can be determined for any specified cosmological theory. The relation is different in different theories, so that in principle it would be possible to distinguish between one cosmological theory and another if we could experiment in this way with a standard object of fixed brightness. Unfortunately it is impossible to move a single object to increasing distances; so the astronomer must rely on similar objects just happening to lie at different distances. The question then arises of how sure we can be that the objects are really similar to each other. Massive galaxies do seem to be quite remarkably similar, but the theoretical differences we are looking for are rather slight in the case of galaxies. This is because galaxies, even the brightest galaxies, cannot be observed far enough away for the theoretical differences to be more than slight. At greater distances the theoretical differences become much more appreciable, however. The technical problem is that of photographing spectrum lines when the light intensity is very small. What is needed is more light. It is here that the quasi-stellar sources are of critical importance. Accepting for the moment that the red-shift of the spectrum lines in these sources is cosmological in character, the quasi-stellar sources are brighter than the most massive galaxies by about four magnitudes, a factor of about forty.

At present, red-shift measurements are available for about fifteen quasi-stellar objects. The shifts are dimensionless numbers given by dividing the wave-length shift of any spectrum line by the laboratory wave-length of the same line. The result is the same for all lines. The measured values range from quite small values, for example 0·16 for 3*C* 273, up to the enormous value of 2 for the source 3*C* 9. The theoretical differences become quite large for red-shifts as great as this, so that a distinction between different cosmological theories should be straightforward once a sample of the order of a hundred quasi-stellar objects has been obtained. The present indication based on the small sample of fifteen is that the universe has expanded from a state of higher density, although the statistical scatter in the sample is large enough to be comparable to the effects that are being looked for.

For spectral shifts as large as two, the Lyman-α line is displaced from the unobservable ultra-violet into the blue, at about 3700 Å. It is possible to look for a continuum on the blueward side of Lyman-α. This continuum is subject to absorption by neutral hydrogen atoms in intergalactic space. A very small density of neutral atoms would be sufficient to absorb out the light completely. Schmidt's observation of 3*C* 9 shows the continuum to be present but to be weakened, that is, to be lower than the continuum on the redward side of Lyman-α. The implication is that intergalactic gas, if it exists, must be hot, perhaps above 10^6 °K. The weakening of the continuum is rather strange, for in a sensitive situation like this one would expect either the continuum to be essentially unweakened or to be absent. The intermediate case seems *a priori* unlikely, since it depends on a closely defined value of the hydrogen density. It is rather in the nature of a coincidence that the density has this critical value.

These remarks are all subject to a cosmological interpretation of the red-shift of the spectrum lines of the quasi-stellar objects. If much smaller, fainter objects were fired out of our own galaxy, or out of some neighbouring galaxy, with speeds close to light the same red-shifts would be observed. Can we be sure that such a 'local' interpretation is wrong ?

Even though on a 'local' hypothesis the quasi-stellars are much less spectacular objects, the total mass involved in all such objects must be as high as $10^6 M_\odot$, or perhaps even more. For such a quantity of matter ejected at speeds close to light, the kinetic energy must be comparable with the rest mass energy, 10^{60} ergs. This is of a similar order of magnitude to the energy involved in the outburst of a major radio galaxy. The energy in the latter case is also in the form of particle motions. The difference is that the particles in the radio galaxies have been thought of in the past as occupying large volumes, not as being condensed into compact objects. However, we now have to ask whether a radio galaxy may not eject compact pieces as well as diffuse clouds of high-speed particles.

Radio galaxies do not eject their material with an initial isotropy. The typical pattern is of two centres of radio emission on opposite sides of a galaxy, with the two centres and the nucleus of the galaxy more or less collinear. To me personally, this has always suggested that an object in the nucleus of the galaxy separates violently into two

* Substance of an Evening Discourse delivered on September 6 at the Annual Meeting in Cambridge of the British Association for the Advancement of Science.

pieces with a large relative motion. The collinear property then follows from conservation of momentum. Exactly the same phenomenon is observed in the quasi-stellar source *MH* 14–121, two regions of radio emission on opposite sides of, and collinear with, a centre of optical emission. The possibility must be considered that a cascade process is involved. An initial object in the centre of a galaxy breaks violently into two pieces. Later, each of these pieces breaks into two further pieces; and so on. As the cascade develops, and as the objects spread out from the parent galaxy, an approximation to an isotropic situation would then gradually develop.

From independent evidence it has been suggested that an explosion occurred in the nucleus of our own galaxy about ten million years ago. If the quasi-stellar objects emerged in this explosion, as Terrell has suggested, the brightest of the objects, 3*C* 273, would now be distant about 0·5 million parsecs, about one-thousandth of the cosmological distance. The optical emission, instead of the enormous cosmological value of 10^{46} ergs sec^{-1}, would be 10^{40} ergs sec^{-1}. The burning of some 300 solar masses of hydrogen gives sufficient energy to supply such an output for as long as ten million years. Since the mass of 3*C* 273 could be set as $10^4 M_\odot$ there would seem to be no difficulty in explaining the optical output. The kinetic energy of 3*C* 273 would then be $\sim 10^{56}$ ergs, about one order of magnitude less than the total energy of the galactic explosion, as estimated by Burbidge and Hoyle. The masses of other quasi-stellar objects can be set lower than 3*C* 273, because 3*C* 273 is intrinsically considerably brighter than the others, at any rate for the sources so far observed. Setting $10^8 M_\odot$ as the mass per object, and taking the mean speed as half the velocity of light, the kinetic energy per object is comparable with that of 3*C* 273. The total energy estimated by Burbidge and Hoyle, 10^{57} ergs, would provide only for ~ 10 objects. It would seem therefore that either Burbidge and Hoyle underestimated the violence of the galactic explosion, or an energy difficulty arises.

The same difficulty does not arise in the case of the galaxy *NGC* 5128. This is a nearby massive elliptical, distant about four million parsec. At least one major outburst is known to have occurred in the nucleus of this galaxy within the past ten million years. The probability must also be considered that both *NGC* 5128 and our galaxy are involved, with our galaxy contributing the comparatively low-speed objects and *NGC* 5128 contributing the high-speed objects.

These questions can undoubtedly be resolved by observation. Observations leading to size estimates for the quasi-stellar objects are coming along with rapidly mounting impetus. The light from 3*C* 273 has been known to be variable in a characteristic time of about ten years. This sets the maximum radius of the optical object at ten light years. In addition, rapid flashes in the light over only a few weeks have been suggested. Largely because the maximum radius would have to be reduced to a tenth of a light-year, or even less, there has been a disposition not to believe this evidence in the case of 3*C* 273. On the cosmological hypothesis, how can one have an optical emission a hundred times brighter than the most luminous galaxies pouring out of an object only a tenth of a light-year in diameter? The issue appears to have been resolved by a recent observation of a doubling of the light of a quasi-stellar source in less than a month.

On the radio side, fluctuations from 3*C* 273 have been found, first by Dent and more recently by Moffet and Maltby. The radio data set stronger constraints than the optical data, particularly for the cosmological theory. The present state of the argument is that the cosmological theory just survives the existing data. Whether it will continue to do so remains to be seen. My judgment of the situation is that survival for the cosmological theory depends on there being a sharp saturation in the accumulation of fluctuation data, that not much more in the way of fluctuations can be tolerated. If we are already near the end of the road, the theory will survive and will then probably turn out the correct theory. But if we are still near the beginning of the road, the prospects for the theory will be slight. I would say we have to do with a fifty-fifty situation.

I would like to turn now to quite different issues; but still bearing on the question I asked at the beginning: Has the universe emerged from a more dense state? The kinds of observation I have discussed so far all relate to great distances. Observations can be made in our own neighbourhood which also bear on the problem. I am now going to describe three such observations, together with the related arguments. The three are utterly different in character, illustrating how wide are the issues in cosmology, and how very many phenomena have to be made to fit into a consistent picture.

Recently, Penzias and Wilson have observed a radio background at a wave-length of about 7 cm, which they do not believe to be due to their equipment or to the nearby terrestrial environment. The intensity is between 10 and 100 times greater than can be attributed to radio sources. The suggestion is that the universe has a thermodynamic radiation background corresponding to about 3·5° K. Observations at two other wave-lengths at least are needed to confirm this suggestion. One such observation is now being planned by Dicke at Princeton.

There seems no way in which such a background can be explained in terms of current astrophysical processes. Hence, if we accept the suggestion of Penzias and Wilson, the immediate implication is that the universe must have been different in the past from what it is to-day. Particularly, a higher density is needed to generate the background.

A similar result follows from the entirely different consideration of the helium-to-hydrogen ratio in stars and gaseous nebulae within our galaxy. Determinations of this ratio range from 0·08 to 0·18; and the ratio seems to be just as high in old stars as in most young stars. The ratio to be expected from current stellar activity is only 0·01. So either activity in the galaxy was much greater in the past, or the helium cannot be explained in terms of production from hydrogen through thermonuclear processes within the galaxy. Failure to observe any stars or any object with a low helium content points to the second of these possibilities.

It is possible to show by detailed calculation that, if matter in the universe has emerged from a state in which the temperature was above 10^{10} °K, the helium-to-hydrogen ratio must be about 0·14, a value which falls in the centre of the observed range. However, no values less than a truly universal value should be found. Two independent determinations for the Sun, one from structure calculations, one from observations of solar cosmic rays, give concordant results close to 0·09. This seems significantly below the expected universal value; but further work is needed to establish whether the discrepancy is real or not.

All the lines of investigation which I have mentioned so far point to an affirmative answer to the initial question: they point to the universal density in the past being higher than it is at present. Yet in every case the argument has been fraught with uncertainty. The probability seems against a negative answer, yet the possibility cannot be excluded. Speaking personally, I believe the case for a negative answer would still be arguable if it were not for the third of my three lines of attack. This again is entirely different in character from the helium/hydrogen ratio and from the microwave observation of Penzias and Wilson. I refer to the problem of the origin of elliptical galaxies. In my view, a consideration of this problem points decisively toward the universe having been very much denser in the past than it is at present.

Galaxies have been broadly classified into two types—ellipticals and spirals. There is an incontrovertible argument to show that spirals must have condensed from a more diffuse form. The spirals are rotating. Their angular

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MATHEMATICS ■ PHYSICS ■ CHEMISTRY ■ BIOLOGICAL SCIENCES ■ MEDICAL RESEARCH ■ SPACE SCIENCES ■ ENGINEERING ● PSYCHOLOGY

momenta prevent them from being compressed into more compact forms. The flattened ellipticals have always been supposed to be similarly in rotation, but this has never been properly checked by observation. Because the ellipticals were thought to be in rotation it was similarly supposed that they were formed by a condensation process. This, I am now convinced, is wrong. I believe ellipticals to have formed through expansion from a higher density state.

Elliptical galaxies are remarkably amorphous. The star distribution is everywhere smooth. If one measures the surface brightness it is found to behave very nearly as an inverse square law, rising with great steepness toward the centre. The centres possess extremely bright central pips. How sharp these centres really are, how star-like, is impossible to say at the moment, for atmospheric seeing effects smear the central pip into an apparent disk.

Suppose the universe expanded from a much denser state, say 10^{-12} g cm^{-3}, and suppose the gas at the beginning of the expansion was not entirely smooth, suppose there were condensation knots already within it. It appears that such condensation knots can restrain the expanding gas to a degree which can be subject to precise calculation. A knot of mass $10^{9}M_{\odot}$ can restrain a total mass of $10^{12}M_{\odot}$ within a region of galactic dimensions. A knot of $10^{7}M_{\odot}$ can restrain a mass of about $5{\cdot}10^{10}M_{\odot}$. The critical point now emerges, that the surface brightness of the resulting aggregation can be calculated (assuming the material forms into stars) and the calculations yield quite unambiguously a law close to the inverse square, in fact just what is observed.

The point I wish to make is that whereas the steep rise towards the centre is expected, and is predicted, by the expansion picture, this characteristic feature of elliptical galaxies cannot, I believe, be understood at all within the condensation picture.

The clinching factor, it seems to me, is that a condensation knot—a memory of the initial dense state—is to be expected at the centre of every major elliptical galaxy. It is these condensation knots that give rise to the phenomenon of the radio galaxy. These are the massive objects which Fowler and I postulated some three years ago. Questions were asked of us at the time as to how our objects ever managed to form. Difficulties of angular momentum were raised. The answer which can now be given is that the objects never formed, in the sense in which the questions were asked. They are relics of a much higher density phase of the universe. They have been there since the galaxies themselves were formed, and in the sense of the radio astronomer they have been smouldering throughout the lifetimes of the galaxies. They are systems which remain at the very edge of stability. Whenever instabilities occur, violent outbursts serve to restabilize them.

Why is my initial question so important? Why make such a fuss about whether the universe has been in a more dense state? Because the present physical theory suggests that there is no limit to how great the density must have been in the past. I use the word 'suggests' because the physical divergence of the density was first demonstrated for a homogeneous and isotropic universe. Divergence also occurs when the isotropic restriction is removed. Does it also occur when homogeneity is removed? I have always believed the answer to this question was also affirmative, but my belief was based more on the failure of those who maintained the opposite to demonstrate their case than on any positive demonstration on the affirmative side. However, progress on the affirmative side has been made very recently, and opinion has generally moved toward the view that the equations of physics contain a universal singularity.

I have always had a rooted objection to this conclusion. It seems as objectionable to me as if phenomena should be discovered in the laboratory which not only defied present physical laws but which also defied all possible physical laws. On the other hand, I see no objection to supposing that present laws are incomplete, for they are almost surely incomplete. The issue therefore presents itself as to how the physical laws must be modified in order to prevent a universal singularity, in other words how to prevent a collapse of physics.

It was with this background to the problem that several of us suggested, some twenty years ago, that matter might be created continuously. The idea was to keep the universe in a steady-state with creation of matter compensating the effects of expansion. In such a theory the density in the universe would not be higher in the past than it is at present. From the data I have presented here it seems likely that the idea will now have to be discarded, at any rate in the form it has become widely known—the steady-state universe. But let me proceed with the theoretical ideas which have grown out of the notions of twenty years ago, for they may turn out to have a value going beyond the first suggestions.

During the past ten years the struggle has been to invent a form of mathematics operating in the manner customary in physics, namely, starting from an action principle. It was found possible to represent the creation of matter through the introduction of a new field. The manner in which the field was treated was quite normal. What was different from ordinary physics was the motive underlying the investigation, the avoidance of a universal singularity, rather than an experiment in the laboratory. Physicists will introduce a new field at the drop of a hat, if experiments in the laboratory should direct them so; but the physicist is unhappy to do so for any other reason.

Having obtained the mathematical structure of the new field it was found that singularities never occur, quite regardless of whether matter is being created or not. So long as the new field exists there will be no singularity either of the universe or of a local imploding body. In other words, the models available for investigation, the models without singularities, were very much wider than the old steady-state theory. During the past few years it is these other models which have been under investigation. What has turned out?

The simplest case is that in which the new field exists but in which there is no creation of matter. It is then possible to obtain a finite, oscillating universe of the kind that has been sought for so long in the usual theory. The universe alternately expands and contracts. Gravitation causes the reversal from expansion to contraction, while the new field causes the rebound from contraction to expansion.

So far as I am aware, such an oscillating model is in satisfactory agreement with all available data. The model is less dull than it seems at first sight, for it contains the possibility of some carry-through from one cycle to the next. Suppose the universe as we observe it eventually stops expanding. Suppose it falls back to a state of comparatively high density, a state in which stars are evaporated, a state in which even the nuclei of heavy elements are disrupted, a state from which matter emerges with the helium-to-hydrogen ratio I described before, about 0·14. In the state of high density things will not be quite uniform. Because of the existence of galaxies, and of clusters of galaxies, there will inevitably be some departures from uniformity. These departures will form the condensation knots round which a new generation of galaxies will form. Thus the condensation knots of which I spoke at an earlier stage are not merely random perturbations. One generation of galaxies acts as the seeds for the next generation. Magnetic fields will also persist from cycle to cycle.

There are two objections to this model. The new field is without sources. It is introduced *ad hoc*, along with the matter. There is never any coupling between the matter and the field. Then there is the subtle, but I believe the correct, objection that a series of oscillations must eventually damp out. Unless dissipative processes are precisely zero,

after a stop of three days at Itacoatiara (about 100 km downstream) to load brazil-nuts, Belém was reached on September 5. Now the party found itself in considerable difficulty as, having come down an international waterway on a British steamer, the chief customs official at Belém held that the party were seeking to re-enter Brazil and questioned the validity of the documents presented. The equipment was impounded and only after a fortnight was the position clarified and the equipment released on September 19, and after a few days it had to be repacked for the journey home. During the stay at Belém the party was almost entirely dependent on borrowed equipment, and it is a pleasure to record that the party was made extremely welcome by Dr. Orlando Costa of the Evandro Chagas Institute, who provided laboratory facilities, and by Dr. Dalcy Albuquerque of the Goeldi Museum, who provided more laboratory facilities and excellent living accommodation in the zoological garden attached to the Museum. If it had not been for the kindness of these two directors no scientific work would have been possible during the time spent separated from the equipment.

Here Walliker had the assistance of the Belém Virus Laboratory in taking blood smears from animals in the Utinga Forest. Garnham turned to the problem of chloroquine-resistant malaria, which had been reported to be present in the area and, with the aid of Dr. Lins of the Evandro Chagas Institute, visited the hospitals and made valuable contacts with physicians dealing with malaria infection. Whether drug-resistant malaria is present or not is a matter of debate among the experts in Brazil, and the matter remains unresolved.

Throughout the visit to Amazonia constant enquiry had been made concerning the possibility of obtaining specimens of the South American lungfish, and in the Manaus region the answers to such enquiries were always that it could be found but not in this precise locality or season. At Belém hopes were higher, for it is well known that it occurs in the island of Marajos which is close by. Eventually a local fisherman was persuaded to try to attempt to find some in one of the many waterways which run through the outlying parts of Belém. These are shallow ditches which are kept full of water by the influence of the tides in the delta; these ditches contain much vegetation (see Fig. 3) and are used by the local inhabitants as places in which to dispose of household refuse and rubbish of all kinds and the water is turbid, of unpleasant odour and, as shown by the electronic recording gear, of negligible oxygen content. At night the fisherman stretched a net across such a ditch and then by working it along the stream and beating the rooted vegetation drove fish into the net. In one night's fishing fifteen specimens of *Lepidosiren* were taken of which twelve were flown back to London in suitable containers. Six were sent independently and six accompanied the members of the expedition on the return journey and all arrived in good condition.

Thus the objective of obtaining living lungfish was attained. What of the other objectives ? It is now, at the time of writing, some ten months since the party returned. During this time work on the material has proceeded and a preliminary scientific report was made at a meeting of the Linnean Society of London on February 18. A series of reports on the parasitological materials, some of which on protozoa are in the press, is being prepared by Walliker, and in a joint report on some aspects of filariasis he is being joined by Garnham. Bishop and Tatchell will make their own reports in due course; Bishop has also been responsible for sending material to experts on various aspects of the work, largely systematic, for their detailed investigation. In addition, a colour 16-mm film recording the expedition and its work is nearing completion. Work on the lungfish is proceeding in the Biology Department at

Fig. 3. The habitat of *Lepidosiren* in Belém, Pará, Brazil

Guy's Hospital Medical School under the guidance of Prof. G. E. H. Foxon.

Of the more indefinite objectives, that of getting to know and working in conjunction with Brazilian scientists has most certainly been achieved. All the members of the party are grateful for the splendid assistance and co-operation received at every turn, not only from those who have been named here, but also from all those whom they met both professionally and socially who helped to make their visit so pleasant; in particular, mention should be made of the British vice-consuls at Manaus and Belém.

In conclusion, the members of the party and all concerned with the expedition thank the many individuals, commercial companies, and public bodies who by their financial support and other aid enabled this journey to be undertaken. Among the public bodies the following should be especially mentioned: the Zoological Society of London, the Medical Research Council and particularly the Wellcome Trust, which by a generous guarantee made the project possible.

I. R. Bishop
G. E. H. Foxon

NUTRITION AND ITS INTERNATIONAL RECOGNITION

By Sir RUDOLPH A. PETERS, F.R.S.

THIS subject has a very long history, but in spite of this the International Union of Nutritional Sciences is not yet accepted as one of the 'international unions' of the International Council of Scientific Unions—even at a time when we all know that a proper knowledge of nutrition is more than ever necessary to save the increasing population of the world from famine. A haphazard use of food does not matter so much when there is plenty, because the body can adapt itself to a badly balanced diet to some extent. It is, however, now vital to know how to use our food with the greatest economy. Many years ago, for example, Chittenden and his colleagues (Chittenden, R. H., *Physiological Economy in Nutrition*, New York, 1904) showed that protein requirements of adults could be much reduced without damage to health.

Why, then, is there delay in accepting nutritional science as a separate science? Probably part of the reason is the complexity of the subject. It involves physics, chemistry, physiology, biochemistry, pharmacology, pathology and medicine, and extends to sociology especially in regard to food habits and the preparation of foodstuffs. Each of these sciences sees only a small part of a complexity. Fifty years ago, nutrition seemed almost synonymous with biochemistry and Gowland Hopkins shared a Nobel Prize with Eijkmann for his part in delineating vitamins, and for showing so clearly that nutrition was a qualitative subject and not just a matter of energetics, the so-called isocaloric value of foodstuffs. This was followed by an outburst of activity in isolating and synthesizing various vitamin factors, in the furtherance of which the U.K. Medical Research Council played a considerable part. For the past eighteen years, however, those concerned with granting finance for research have turned to 'pastures new', witness: The isolation of enzymes, the unravelling of protein structure and the excitement of the nucleic acids and genetics. The result is that at the present time few research workers have been trained in the rigorous and lengthy discipline of nutritional research—which is emphatically not just 'the feeding of a few rats'. From many points of view, even from that of space travel, we want to know much more about the interaction of the various compounds making up a diet.

The problems extend, of course, beyond man into the animal kingdom. In agriculture, for example, there is the problem of digestion in the ruminant. The micro-organisms in the rumen, bacteria and protozoa, by a controlled fermentation set up a digestion of substances such as cellulose, which the secreted enzymes of the digestive tract cannot digest; and so they turn inedible substances into food for man. Anatomy, physiology, biochemistry and microbiology are all involved in this work; but none of these sciences, as such, is interested in the implications of ruminant digestion for the utilization of the world's grassland to feed its populations.

Again, it is the business of the soil scientist, who may be geographer, physicist and chemist, to show that certain strata of the world's surface lack particular elements, such as copper or cobalt; and the biochemist will relate this to plant growth and to enzymes present. It is the business of the nutritionist to relate these discoveries to the health of the grazing animal, and so ultimately to the food supply of populations. Actually, it has often been investigated in reverse, starting with the ill-health of the animal on the natural vegetation.

It is to be noted that the International Union of Nutritional Sciences has been in existence since 1948; and it has at present the support of seventeen national members of the International Council of Scientific Unions. At intervals of three years, it has held six successful international congresses; the last one was in Edinburgh under the presidency of Sir David Cuthbertson. There will always be a need for close relations with organizations such as the Food and Agriculture Organization, the World Health Organization and Unesco; but this very necessity makes it all the more important for nutrition to have a firm scientific basis within the organization of the International Council of Scientific Unions.

NEWS and VIEWS

Science Research Council Support for University Science and Technology

The Science Research Council has set up a new University Science and Technology Board under the chairmanship of Sir Ewart Jones, Waynflete professor of organic chemistry in the University of Oxford, to advise on the support which the Council should give to science and technology in the universities. The new Board will advise the Council on the award of grants for the support of university research, as well as studentships and fellowships for the training of young postgraduate scientists: support for university scientific research and advanced training will thus be closely integrated. The scope of the Board will include science and technology; it will therefore work in close touch with the other Research Councils and the Ministry of Technology. The Council has already set up a Nuclear Physics Board under the chairmanship of Prof. C. F. Powell, and an Astronomy, Space and Radio Board under the chairmanship of Sir Bernard Lovell. Part of the support previously given to university research and training by the Department of Scientific and Industrial

Research, namely that concerned with earth sciences and ecology, has been taken over by the Natural Environment Research Council; and most of the support given in the past by the Department of Scientific and Industrial Research to university research and training in some parts of the human sciences (particularly sociology and psychology applied in industry) will be taken over by the new Social Science Research Council which is being set up by the Government following the recommendation of the Heyworth Committee.

The new Board replaces the Research Grants Committee and the Postgraduate Training Awards Committee of the Department of Scientific and Industrial Research. The membership of the Board is as follows: *Chairman*, Sir Ewart Jones. *Members*, Prof. B. Bleaney, Dr. Lee's professor of physics, University of Oxford; Prof. R. Brown, Regius professor of botany, University of Edinburgh; Dr. A. Caress, Imperial Chemical Industries, Ltd.; Prof. G. C. Drew, professor of psychology, University College, London; Dr. H. M. Finniston, International Research and Development Co., Ltd.; Prof. H. Ford, professor of applied mechanics, Imperial College of Science and Technology; Prof. M. R. Gavin, professor of electronic engineering, University College of North Wales, Bangor; Lord Halsbury; Dr. J. W. Menter, Tube Investments Ltd.; Prof. R. S. Nyholm, professor of chemistry, University College, London; Prof. G. Porter, Firth professor of chemistry, University of Sheffield; Prof. E. J. Richards, professor of applied acoustics, director of Institute of Sound and Vibration Research, University of Southampton; Prof. A. L. Roberts, Livesey professor of fuel technology, University of Leeds; Dr. J. E. Smith, secretary of the Marine Biological Association of the United Kingdom and director of the Laboratory; Prof. I. N. Sneddon, Simson professor of mathematics, University of Glasgow; Sir Peter Venables, vice-chancellor, University of Aston in Birmingham. *Assessors*, University Grants Committee and the Ministry of Technology.

Directorate of Overseas Surveys : Mr. G. J. Humphries, C.M.G., O.B.E.

Mr. G. J. Humphries retires this month as director of Overseas Surveys of the Ministry of Overseas Development, and Surveys adviser to the Minister. Mr. Humphries was born in 1900, and after three years at the University of Reading joined the Colonial Survey service in Nigeria in 1928. He was with the Royal Engineer Survey from 1939 until 1946, attaining the rank of lieutenant-colonel. From 1944 he was responsible (under Brigadier Hotine, director of Military Surveys) for the planning and organization of what was originally known as the Directorate of Colonial Surveys. Mr. Humphries was deputy director of this from its start at Bushy Park in 1946 with responsibility for the field side of the work. He was appointed director in 1963 on the retirement of Brigadier Hotine. When the tellurometer was being perfected in South Africa in 1957 he played a major part in its acceptance by geodesists and land surveyors by his advocacy and by organizing the completion by tellurometer in one month of a 400-mile precise traverse in Kenya which would have taken two years or more by conventional methods—the first major field task for which this revolutionary survey instrument was used. In addition to his control of the field side of the Directorate with parties working all over the Commonwealth, Mr. Humphries was responsible for the technical side of the selection and training of British surveyors for the Colonial Survey departments and of the training of African and other overseas surveyors in Britain. His experience and judgment of character have played an important part in the growth of these departments and in their effective evolution after the independence of the countries concerned. Mr. Humphries was awarded an O.B.E. in 1954 and a C.M.G. in 1965.

Mr. W. D. C. Wiggins, O.B.E.

Mr. W. D. C. Wiggins has been appointed to succeed Mr. Humphries as director of Overseas Surveys and Surveys adviser. Born in 1905, Mr. Wiggins was a scholar of King's School, Canterbury, and went to University College, London. He joined the Nigerian Survey Department in 1928 and served in the Royal Engineers from 1939 until 1946, attaining the rank of lieutenant-colonel. He was appointed assistant director to the Directorate on its formation in 1946 and deputy director in 1948 with responsibility mainly for the mapping side at Tolworth. He was awarded an O.B.E. in 1957.

Standards Division, National Physical Laboratory, Teddington : Dr. H. Barrell, C.B.E.

Dr. H. Barrell, who will be retiring from his post of superintendent of the Standards Division of the National Physical Laboratory at the end of March 1966, joined the Metrology Division, as it then was, in 1923. He took his degree in physics at the Royal College of Science, where as a demonstrator he later worked with A. Fowler on ultra-violet spectroscopy in the early years of quantum theory. Dr. Barrell's own experimental work has been largely devoted to precise measurements of wave-lengths in the visible spectrum and their applications to the measurements of length. An outstanding contribution, made jointly with Mr. J. E. Sears during 1932–38, was the determination of the metre and the yard in wave-lengths of light and of the refractive index and dispersion of air. From 1939 until 1945 he was responsible for mechanical gauging of ammunition to which he applied pneumatic methods, so initiating an important development in the work of the Division. Dr. Barrell was appointed superintendent of the Metrology Division in 1953 in succession to Dr. F. H. Rolt. In 1958, in a re-organization of the National Physical Laboratory, the Metrology Division was combined with other groups to form the Standards Division. Dr. Barrell has been particularly concerned with the decision, taken in 1960, to define the metre in terms of the wave-length of an orange line in the spectrum of krypton-86: he has directed the Division's contribution to the international co-operative programme of research organized by the International Committee for Weights and Measures and he is a member of that Committee's Advisory Committee for the Definition of the Metre. In 1954, following the death of Mr. J. E. Sears, he was elected to the International Committee. He was a member of the British delegations to the General Conferences of Weights and Measures of 1948, 1954, 1960 and 1964. As chairman of the international Advisory Committee for the Definition of the Second, Dr. Barrell has been influential in securing agreement on the definition of time in terms of an atomic frequency, a decision which, like that on the metre, depends heavily on the experimental work of his own Division. Dr. Barrell's superintendency has been a time of rapid change. From a small group concerned mainly with measurements in classical physics and engineering, the Division has grown to be the largest in the Laboratory with responsibility for the fundamental units, for the most precise measurements of length, mass, time and frequency, electrical quantities and temperature, and for much of the routine test work of the Laboratory as well. This growth Dr. Barrell has taken in his stride while maintaining and encouraging the many research activities and manifold international responsibilities of the Division. He retires at a time when the international repute of the Division is high in the field, a true reflexion of the imaginative guidance he has given. He was awarded a C.B.E. in 1962.

Dr. A. H. Cook

Dr. Barrell is being succeeded next April by Dr. A. H. Cook, who was educated at Westcliff High School and Corpus Christi College, Cambridge. After graduating

in physics in 1943, Dr. Cook worked at the Admiralty Signal Establishment on electrical measurements at micro-wave frequencies and returned to Cambridge in 1946 to specialize in the Department of Geodesy and Geophysics on pendulum and gravity meter surveys of the gravitational field in Britain and continental Europe. He joined the staff of the National Physical Laboratory in 1952 and has gained international renown for his outstandingly accurate determinations of fundamental quantities, in particular the density of mercury, wave-lengths of light and gravitational acceleration at Teddington, which are of basic importance in the establishment of more precise standards of measurement. During recent years his interests have extended to metrological applications of gas lasers and to the development of advanced methods and equipment for interferometric spectroscopy in the extreme ultra-violet region. He is planning, in association with the University of Trieste, a new determination of the gravitational constant, using for the experiment a torsion pendulum 80 m long suspended in a large cave near Trieste. Dr. Cook is a member of the British National Committees for Geodesy and Geophysics and for Space Research and holds official positions in the International Union of Geodesy and Geophysics and the International Astronomical Union. He has served on the Council of the Royal Astronomical Society and is joint editor of the *Geophysical Journal*. He is now being granted sabbatical leave until he takes up his new appointment and will be a Visiting Fellow at the Joint Institute for Laboratory Astrophysics at Boulder, Colorado, which is directed by the University of Colorado and the American National Bureau of Standards.

U.S. National Bureau of Standards : Dr. W. J. Youden

Dr. William J. Youden has retired from the Applied Mathematics Division at the National Bureau of Standards. Dr. Youden, who had been with the Bureau since 1948, was responsible for significant research in mathematical statistics, especially in the field of experiment design, and vigorously promoted sound understanding and increased utilization of modern statistical techniques throughout science and industry. His most outstanding achievements are in the field of combinatorial arrangements for the conduct of scientific experiments. One class of these designs, constructed for use when test conditions vary from one group of tests to another, is known as the Youden Square. He has also contributed methods for interlaboratory tests that are used in biological and medical applications as well as in the physical sciences. Before joining the Bureau staff, Dr. Youden carried out scientific and statistical research at the Boyce Thompson Institute for Plant Research, Inc. He was an instructor at the University of Rochester, and has had temporary professional appointments at Columbia University, the University of North Carolina, and the University of Chicago. Born in Australia, Dr. Youden received a B.S. in chemistry, mathematics, and engineering from the University of Rochester in 1921 and an M.A. and Ph.D. from Columbia University in 1924 in chemistry. Dr. Youden plans in the future to take up a teaching position in the College of Engineering at the George Washington University.

Dr. P. H. Verdier

Dr. Peter H. Verdier recently joined the staff of the Molecular Properties Section, Polymers Division, of the Institute for Materials Research of the National Bureau of Standards as a physical chemist. He will undertake research of the dynamics of polymer chains by means of the Monte Carlo system, and will specialize in the effects of excluded volume interactions on the motion of polymer chains. Dr. Verdier joined the Bureau from the Union Carbide Research Institute where he was engaged in research on molecular structure and dynamics. Born in 1931 in Pasadena, California, Dr. Verdier received his B.S., with honours, in chemistry from the California Institute of Technology in 1952. In 1957 he received his Ph.D. in physical chemistry from Harvard University. He was a Research Associate in chemistry at the Massachusetts Institute of Technology during 1957–58, and a Research Fellow at Harvard during 1958–59.

Chemistry at Battersea College of Technology : Prof. J. A. Elvidge

Dr. J. A. Elvidge has been appointed to the second chair in the Department of Chemistry at Battersea College of Technology, the proposed University of Surrey. Educated at Haberdashers' Aske's Hampstead School (now at Elstree) and the Imperial College of Science and Technology, he graduated with honours in 1943 and gained the Hofmann Prize. As a William Gilles and then Beit Fellow, he carried out research on penicillin in a team under Sir Ian Heilbron and Dr. A. H. Cook, which gained him a London Ph.D. in 1946. Appointed lecturer at the (then) Royal Technical College, Glasgow, in 1947, he worked with Prof. F. S. Spring on gliotoxin and aspergillic acid. On returning to the Imperial College of Science and Technology in 1949, he collaborated with (then) Prof. R. P. Linstead in research on unsaturated acids and lactones, and macrocyclic compounds and intermediates, and took charge of the newly instituted third-year course for specialists in organic chemistry, which remained his major and expanding teaching interest. He was promoted to senior lecturer in 1956 and to a University readership in organic chemistry in 1962, and at this time received a London D.Sc. Dr. Elvidge's research interests include heterocyclic syntheses from malonyl chloride, reactions of nitrogen heterocycles, tetrazaporphins, metal derivatives, and applications of proton magnetic resonance to structural problems and in the determination of aromaticity. He has collaborated with chemists at the Brewing Industry Research Foundation on the structure and enolization of acyl*cyclo*pentanone products from hop resin. He is a consultant to Fisons Pest Control, Ltd., and prizes close relations with Imperial Chemical Industries Dyestuffs Division. He has been a lecturer and tutor at the recent summer schools on nuclear magnetic resonance spectroscopy organized by the Royal Institute of Chemistry. In his new post, Dr. Elvidge looks forward to introducing nuclear magnetic resonance techniques to an established research school, to further contacts with industry, and participation in the arrangements for the proposed new University at Guildford.

1965 Guggenheim International Astronautics Award : Prof. M. V. Keldysh

The Daniel and Florence Guggenheim International Astronautics Award for 1965 has been conferred on Prof. Mstislav V. Keldysh, of the U.S.S.R. Academy of Sciences in Moscow. Prof. Keldysh has made valuable contributions to the progress of astronautics and he is well known for his extensive research in problems of aerodynamics, fluid mechanics, rocket propulsion, and vibration theory. For several years he has been directing work on rocket propulsion and the mechanics of space flight. Prof. Keldysh has been president of the U.S.S.R. Academy of Sciences since 1961 and is also a member of the Praesidium of that Academy's Committee on Theoretical and Applied Mechanics and of the editorial boards of several scientific journals. He has received numerous awards in recognition of his achievements. He is a Corresponding Member of the Engineering Sciences Section of the International Academy of Astronautics. The Guggenheim International Astronautics Award, which is offered by the International Academy of Astronautics, carries with it a prize of 1,000 dollars.

U.K. Atomic Energy Authority and Central Electricity Generating Board Co-operation Scheme

To facilitate the close co-operation which already exists between the United Kingdom Atomic Energy Authority and the Central Electricity Generating Board, it has been decided that there should be cross-membership between the two bodies. Mr. F. Cousins, Minister of Technology, has accordingly appointed Mr. E. S. Booth, the engineering member of the Central Electricity Generating Board, to be a part-time member of the Atomic Energy Authority, and Mr. F. Lee, Minister of Power, has appointed Mr. J. C. C. Stewart, the Member for Reactors of the Atomic Energy Authority, to be a part-time member of the Central Electricity Generating Board.

Professional and Technical Staff in the Civil Service

In a recent issue of *State Service*, the journal of the Institution of Professional Civil Servants (August 1965), the general secretary, M. W. McCall, commenting on the 1964 report of the Civil Service Commission, stresses the continuous failure to recruit sufficient professional and technical staff. Candidates could only be recommended for about a third of basic grade vacancies for engineers, although two-thirds of the vacancies at main grade and higher levels were filled. Similarly, while two-thirds of the vacancies for architects in these grades were filled, only one-sixth of those at the basic grade were filled. Only 85 out of 220 vacancies for mechanical and electrical engineers were filled and only 327 out of 546 vacancies in the technical classes. The Board of Inland Revenue is stated to be short of a fifth of its complement of professional valuers, the work of the Patent Office is delayed for a like reason, and the shortage of Factory Inspectors has been reflected for years in the low frequency of inspections. To remedy this situation, Mr. McCall urges that pay and careers must be offered to scientists, technologists and technicians which indicate that they are no longer regarded as inferior or second- and third-rate. Their training and talents should be used to the field in terms of the work they are qualified to do and they should have full opportunity of promotion to the highest posts.

Aid to Education Overseas

The Overseas Development Institute has issued the report of a Ditchley Foundation Conference held at Ditchley Park during March 26–29, 1965 (*Aid to Education: An Anglo-American Appraisal*. Report of a Ditchley Foundation Conference held at Ditchley Park, March 26–29, 1965. Rapporteur: Peter Williams. Pp. 52. London: The Overseas Development Institute, in association with The Ditchley Foundation, 1965. 3*s*. 6*d*.). Dealing with educational aid, especially in Africa, Asia, and the Caribbean, it considers first the donor programmes and policies of educational aid, and second the requirements of the recipient countries. Next it sets forth some conclusions about priorities and the principles of educational aid before making specific suggestions for future Anglo-American co-operation in this field. Recognizing the need for a balance between education and the economy, the Conference gave much thought to the determination of priorities between types and levels of education. It also emphasized that educational aid is a two-way process and that the developing countries should not copy too rigidly the institutions and techniques of developed countries. It is emphasized that the developing countries will need educational assistance for a very long time ahead. The Conference strongly urged that Britain and the United States should, as the major donor countries, prepare themselves to meet the challenge by establishing permanent resource centres on which to draw in their aid programme.

It suggests that it would be wise to establish small cadres of experts on a permanent footing and to create supernumerary posts in Government departments, universities and other institutions. Moreover, appropriate arrangements should be made for training the personnel involved, so that specialists could be seconded for overseas assignments as required. In this way a permanent supply of experts would be ensured. The advantages of involving non-government institutions are also emphasized, as well as the need for a strategy of educational aid. It is suggested that existing forms of co-operation, like the Teachers for East Africa Scheme and programmes of joint support for overseas universities, should be extended and supplemented by new schemes. Specific areas for future co-operation should include the development of curricula and text-books, the teaching of English, application of modern techniques (like educational television and programme learning), educational research, the training of teachers, experiments in comprehensive secondary school education and work on methods of reducing the costs of education. Pilot projects and experiments should be financed so far as possible by aid donors. Direct linking of American, British and overseas teacher-training institutes for various purposes is recommended as well as co-ordination of British and American programmes for teacher refresher courses in developing countries.

Water Affairs in Zambia

The independence of Zambia came about in 1964 and inevitably brought with it great changes in the administration and conduct of the work of official organizations concerned with development of natural resources, including water. The Department of Water Affairs had to adjust itself to the new conditions, and how it set about this task is revealed in the recently published annual report of the Department of Water Affairs for the year 1964 (Pp. 9. Lusaka: Government Printer, 1965. 2*s*.). One of the major difficulties which had to be faced was the loss to the Water Department of many well-trained and experienced members of the professional and technical staff who decided to leave the country and seek a new life elsewhere. The report devotes considerable attention, and rightly so, to the serious staff position thus created. Notwithstanding this handicap, and the rather alarming number of vacancies existing in the various grades of water engineers and technical staff, actually 39 at the close of 1964, the amount of development work accomplished in many areas now under regional planning, including investigation and construction of new water works, appears quite remarkable. Special mention is made of the work of the Hydrological Branch in respect of the Kafue Basin Survey, where an investigation of the river system was considerably extended by installation of many new stations chiefly in the area between Mumbwa and the Copperbelt. Good progress was also made in rehabilitation of old drainage canals and also in construction of new canals in Barotseland. It is recorded that there has since been a notable improvement in drainage of areas affected by these works; crops can now be grown where formerly the land was far too wet. Further sections of the report summarize the present position of the Copperbelt water supply; other work of the Hydrological Branch; water projects in view; water communications and drainage in the Bangweulu Swamps; drainage and communications in Barotseland; the functions of the Water Board and of the Geological and Drilling Sections.

Scientific Research in the Development of Polish Industry

In the *Review of the Polish Academy of Sciences* for January–March 1965, I. Malecki describes the role of scientific research in the development of Polish industry since the Second World War. The period 1945–52 was

largely one of reconstruction. During 1953–57 the technical base was expanded, and this period was marked by an extension of the industrial institutes, which in 1957 numbered 79, employing 22,600 scientific and technical workers, compared with 1,000 in the research institutes of the Polish Academy of Sciences. The next period, 1958–63, was notable for modernization and a general raising of the level of production, and has since been followed by one of systematization and development of the work of the research centres. Throughout these phases, Dr. Malecki emphasizes the attention given to the exploitation of natural resources and to the creation of the technical infra-structure; to improving the properties and production of novel technical materials; to the design of buildings and of major technological processes; and to the development of electronics and machine production. The situation of the medical sciences in Poland is described by K. Rowinski in a further article in the same issue. The physiological sciences are still inadequately developed in Poland, but the pharmacological and pharmaceutical sciences have contributed considerably to the supply of drugs. In biochemistry developments have been more in fundamental research than in the clinical field. Valuable work has been done in histology and in medical microbiology, and the clinical sciences are stated to be progressing favourably.

The Pharmaceutical Industry

In recent years the pharmaceutical industry in Britain, and, for that matter, overseas also, has been the target for much adverse criticism on several counts, notably high prices of some new drugs, inadequate clinical testing of newly marketed products, the problem of 'side effects', and a belief that profits in the industry overall are excessive and nationally unwarranted. That much of this criticism is ill-informed and unjustified is made clear in two recent publications which are worthy of a much wider circulation than they will probably achieve. The first is *Astra: Annual Report 1964* (Pp. 40. Södertälje: Astra, 1965). This is in English and. in the course of his annual review, the President of the Astra Group says: "During the year there was a continued trend in most countries toward more rigorous legislation for pharmaceutical products. Behind this is the urgent desire to improve safety conditions for the consumer. However, the divergent regulations in various countries and the growing bureaucratic interference create great difficulties for the industry and delay the introduction of new drugs, yet have failed to ensure a corresponding degree of safety. Efforts are being made to produce more unified rules governing new drug registration". A much more detailed exposition of the problems of the industry to-day appears in a recent issue of *CIBA Journal* which, in a series of impressive articles lavishly illustrated, is devoted to the subject (33. Pp. 62. CIBA Ltd., Basle, Switzerland; Spring 1965). This is again in English and the titles of the articles are self-explanatory. The editorial foreword discusses at some length "The Intricate Mechanism of Drug Discovery, Manufacturing, and Control". R. Larose (CIBA Dorval) details something of "The Canadian Pharmaceutical Industry at Work". "The Viability of the British Pharmaceutical Industry" is ably revealed by A. W. Morrison (CIBA Horsham). An anonymous article entitled "Working in the Light: The New Drug Research Unit at Horsham" describes the modern building there now at the disposal of those engaged in the search for new and better pharmaceuticals (*Nature*, 207, 236; 1965). Prof. H. Bloch (CIBA Basle) writes on "The High and Rising Importance of Biology in Pharma Research". Dr. Matthys Staehelin (CIBA Basle) contributes an account of "Biochemistry—Cornerstone of Biological Research". It is impossible to read these articles without coming to the conclusion that, in spite of sins of omission and commission such as there may have been in the past, the international outlook of the industry to-day is to work for the betterment of mankind and to spare neither energy nor money in giving the medical profession the tools it needs to alleviate human suffering. One gets the impression that Government interference in what is after all pure scientific research, if carried to excess, must inevitably defeat its own object. Freedom to explore, to formulate, to think ahead, untrammelled by obstructive regulations, is the privilege of every qualified worker in fundamental scientific research; in no case is this freedom more vital to the community than in the practice of pharmacology.

Load-bearing Brickwork

At a meeting of the Building Materials Section of the British Ceramic Society held at the Building Centre, Store Street, London, during November 18–19, 1964, nine papers were presented dealing with some fundamental properties of structural brickwork. These have now been published under the collective title *Load-Bearing Brickwork* (*Proc. Brit. Ceramic Soc.*, No. 4. (July 1965): *Load-Bearing Brickwork*. Pp. 121. Stoke-on-Trent: British Ceramic Society. 25*s*.). The first paper, by D. Lenczner, is on "Design of Creep Machines for Brickwork" and deals with the creep characteristics of brickwork and particularly the various creep machines designed for testing brick piers, walls and brick mortar. Next, M. E. C. Stedham contributes results of his further work to establish crushing tests of 9-in. brickwork cubes as an indication of the strength of brickwork built of the same materials, under the title of "Quality Control for Load-Bearing Brickwork". The third paper entitled "Measurement of Strains in Load-Bearing Brickwork", by R. N. Picken and A. W. Hendry, describes the construction, mode of use, and application of the vibrating-wire strain-gauge to the measurement of strains in the brickwork at the base of a brick chimney during dismantling at Hem Heath Colliery, Stoke-on-Trent. J. M. Plowman in "The Modulus of Elasticity of Brickwork" compares the elastic moduli obtained from brickwork made from bricks and mortar of wide ranges of strength. "Comparative Tests on $\frac{1}{3}$- and $\frac{1}{8}$-scale Model Brickwork Piers and Walls", by A. W. Hendry and C. K. Murthy, describes their investigations carried out to ascertain the suitability of model bricks; tests that had been carried out on full-size brick piers at the Building Research Station and on full-size walls at the Department of Building Science, University of Liverpool, were repeated on $\frac{1}{3}$- and $\frac{1}{8}$-scale models; their results show that, for given strengths of brick and mortar, subject to certain provisos, the strength of full-size brickwork can be reproduced with reasonable accuracy by means of model tests. The sixth paper by S. Prasan, A. W. Hendry and R. E. Bradshaw, entitled "Crushing Tests on Storey-height Walls 4·5 in. Thick", discusses the results of tests on seventeen walls 4·5 in. thick loaded between reinforced concrete slabs and their significance in terms of the various mechanical factors involved. A paper by L. G. Simms on "The Strength of Walls built in the Laboratory with some Types of Clay Bricks and Blocks" briefly describes loading-tests on walls of storey-height built in the laboratory (Building Research Station) with clay bricks and blocks. F. H. Clews discusses "Experiments to Assess the Durability of Bricks and Brickwork": an account of natural exposure tests, with examples of techniques applied by other workers, and of the methods used at the Mellor-Green Laboratories on fifty-nine batches of bricks and thirty-two batches of roofing tiles. Finally, a paper entitled "The Design and Economics of High Load-Bearing Brick Structures", by R. A. Sefton-Jenkins, dealing with the design of highly stressed brickwork, includes comparison of various codes of practice with the British, discussion of economics of load-bearing brickwork compared with other structural forms, and a plea for more research into this subject as a basis of a new British Code of Practice.

Thermal Comfort in Industry

In everyday circumstances of industrial and, for that matter, domestic life, the range of temperature and humidity that can be tolerated by the human body is really quite small. Human reaction to different thermal conditions, especially in factories and offices, is a subject of research falling within the ever-widening scope of ergonomics. In this context, there has recently been published a booklet by Dr. R. H. Fox, of the Division of Human Physiology, Medical Research Council (Ministry of Technology. *Ergonomics for Industry*, No. 8: *Thermal Comfort in Industry*. Pp. 52. London: H.M.S.O. Obtainable from Warren Spring Laboratory, Stevenage, Herts. 1965. *Gratis*). The essential theme of this guide-book (for that is the underlying purpose of each issue in the *Ergonomics for Industry* series so far available) is not only a discussion of human reactions to environmental conditions of temperature and humidity, but also the ways and means of ensuring that optimum conditions may be established. Quoting one specific instance (well illustrated), the author says: "Few people would choose to live in a greenhouse all the year round, yet the indoor climate in this modern office building is not very different". Thus attention is directed to the different conditions that different types of work require. It is recommended that buildings should be planned with the comfort and working efficiency of the occupants in mind. It stresses the problems encountered in some modern office buildings where large areas of glass prevail, with relatively little, if any, provision for air conditioning; such conditions result in overheating and consequent fatigue in summer, and coldness and discomfort in winter. Either of these extremes is inimical in the long run to welfare and efficiency. It is admitted that in some cases extremes of temperature occur and thermal control is impracticable; in the event, protective clothing and training are necessary; the principles involved in insulating individuals in such environments are outlined. The illustrations in this booklet are impressive, so is the advice and practical guidance throughout the text. Suggestions are made for further reading on this important subject of human and environmental relationship, while sources of specialist advice, and information on general ergonomic questions and on heating, ventilation and insulation of buildings, conclude a most practical exposition of the problems involved.

Science Information and Documentation Services

A *World Guide to Science Information and Documentation Services*, issued by Unesco, is intended primarily as a guide to the location of such services dealing with the natural sciences (Pp. 211. Paris: Unesco, 1965. 9 francs; 13*s*.; 2.50 dollars). Technology is to be dealt with in a separate volume, but services for agriculture and medicine are expressly included because of their paramount importance for the developing countries. The *Guide*, which is in both French and English, is based on 403 completed forms returned in a detailed questionnaire, and covers 144 institutions representing 65 countries. The institutions are listed under countries in alphabetical order of their titles and the entries indicate the subjects covered, approximate holdings and services offered. There is also an alphabetical list of institutions and a subject index. It might be noted that there are only three entries for the United Kingdom: Aslib; the National Lending Library for Science and Technology; and the Science Museum Library.

British Art, 1740–70

The American Philosophical Society has rendered a notable service to art-history by publishing Prof. E. K. Waterhouse's Jayne Lectures for 1964 under the title *Three Decades of British Art, 1740–70* (*Memoirs of the American Philosophical Society*, 63. Pp. xii+77. Philadelphia: American Philosophical Society, 1965. 2 dollars). But these discourses can be interpreted more widely than that, namely, as a conspectus of what a certain cross-section of society was like at that period. In general, we note the advent of Italian influence, culminating in the Grand Style, and with it the works of such masters as Hogarth, Reynolds and Gainsborough. To men of science, there are several points of interest, but two stand out clearly. One is the statue of Handel by Roubiliac (illustrated), so complex and rather voluptuous, when one recollects that it is by the same hand as the ascetic and sublime "Newton" in Trinity College Chapel. How versatile and sympathetic the sculptor must have been to respond like that in his portrayal of two utterly different characters. The second matter is the 'characatura' (by Reynolds) of Raphael's *School of Athens*. This is indeed an odd fantasy—the great Greek philosophers and mathematicians replaced by eighteenth-century milords and their fellow-travellers, seen against a background of Gothic niches (in place of Raphael's classical statues). Sir Joshua was far too great a man to perpetrate this merely for fun. He was showing up his contemporaries, and incidentally instructing himself quite effectively. Altogether a most intriguing episode, described with exceptional insight.

University of Leicester, Department of Museum Studies

With the assistance of a £15,000 grant from the Gulbenkian Foundation, the University of Leicester has established a postgraduate Department of Museum Studien. The University has appointed H. R. Singleton, at presest director of the City Museum, Sheffield, to be the first director of this new Department. His duties will commence on January 1, 1966, and it is expected that the first students will be admitted in the following October. Initially, the intake will be confined to graduates with qualifications in archaeology or geology, but in subsequent years it is hoped to accept graduates from other disciplines. The one-year, full-time course for a Certificate in Museum Studies will be designed to offer intending entrants to the museum profession a preliminary training in museum administration and organization, in the museum application of their particular subject, and in the practical side of museum work.

National Museums of Southern Rhodesia

The report of the Trustees and Directors of the National Museums of Southern Rhodesia for the year ended December 31, 1964, records the probably unique event of the opening of three new museum buildings within one year, namely, the National Museum, Bulawayo, the Queen Victoria Museum, Salisbury, and the Umtali Museum (Pp. 25. Salisbury: National Museums of Southern Rhodesia, 1965). With the future in mind, each of the buildings is designed to allow for expansion, and the sites have sufficient ground reserved for extension. Although the fittings in the exhibition galleries were far advanced for the opening ceremonies, much still remains to be done. This great development has come at a time when political considerations in Africa have disturbed the progress which took place in the immediate post-war years. As a result, funds are not so freely available as formerly, and so, with Government sanction, it was decided to levy an entrance charge to help to offset the greatly increased maintenance costs on buildings some two-and-a-half times the size of the old. In view of the educational nature of the organization, school parties are admitted free.

Meat from Wild Animals in Africa

In the past decade increased attention has been directed towards the management and potential utilization of wild

animals in Africa as a natural resource in order to assist in filling the world's larder. The more scientific investigational work which has been carried out in this field has been brought together by the Commonwealth Agricultural Bureaux in a recent publication, issued as *Technical Communication* No. 16, entitled *The Meat Production Potential of Wild Animals in Africa—A Review of Biological Knowledge* (By Lee M. Talbot, W. J. A. Payne, H. P. Ledger, Lorna D. Verdcourt and Martha H. Talbot. Pp. vii+42. Farnham Royal, Bucks.: Commonwealth Agricultural Bureaux, 1965. 13*s*. 6*d*.). This directs attention to the possibilities of exploiting some of the game animals in under-developed countries. The review points out that the African continent contains the greatest diversity of indigenous large mammals to be found anywhere in the world. The wild ungulates considered range from the 10-lb. dik-dik to the 5-ton African elephant. The determination of the numbers of animals present in an area has represented a major problem. In general, the most accurate estimations were made from light aircraft, and the results agreed closely with those obtained by Royal Air Force photo-reconnaissance planes. Some animals were marked so that they could be recognized later and their movements and growth-rates followed. Agriculturists and meat technologists will be interested in the figures given under the heading "Carcass Composition". Killing-out percentages quoted include the following: East African zebu steers, 40 to more than 60; wildebeest, 50; topi and kongoni, 53; and impala and gazelle, both about 60. In general, the amount of fat is consistently low, so that the carcass yield is relatively constant within a given species.

Soil Catenas

Since Milne used the word 'catena' as a mapping unit in East Africa, some thirty years ago, to describe a sequence of soils, extending from hill top to valley bottom, which is repeated across the contours of the landscape, it has been interpreted in various ways in different countries. Such matters as parent material, erosion, intensity of leaching and mobility of sesquioxides have tended to complicate the original concept, which was intended to apply to one climatic zone, a medium-rainfall area of the tropics, and to differences in level. It is most useful, therefore, to have a documented statement on the subject by J. P. Watson in *Soils and Fertilizers* (28, No. 4, 307; 1965). The same number contains a review by Dan H. Yaalon of a Russian book on *Soil Geographical Zoning* of the U.S.S.R., and a description by W. E. Calton of a soil-map of Africa (scale 1 : 5,000,000) with an explanatory monograph by J. L. d'Hoore. The former is a systematic inventory of the natural soil and climatic resources of the U.S.S.R.; the latter is a large inventory of the soils of Africa with seven sheets of soil maps.

Soil Survey of Great Britain

The work completed and in progress during 1964, under the auspices of the Soil Survey Research Board of the Agricultural Research Council, is described in its seventeenth report (Pp. v+46. London: Agricultural Research Council. Obtainable from H.M.S.O. 1965. 6*s*. net). Publications include a memoir on "The Soils and Land Use of the District around Aylesbury and Hemel Hempstead", two bulletins on the soils of the Middle Teign Valley and of six counties in the West Midlands, and a soil map of 432 square miles (sheet 57) around Forfar. Two more memoirs on the Mendip District and on the country around Haddington and Eyemouth, and eleven sheets of soil maps, are in the press. Members of staff of the Soil Survey also contributed 21 papers to scientific journals and conferences. This report contains short accounts of the nature of the soils found in various parts of Britain, with emphasis on unusual features, and some classifications of soil series. There is also a summary of the laboratory investigations that have been carried out. The Soil Survey is frequently called on to make special surveys, and the assistance rendered to such authorities as the Forestry Commission, the Nature Conservancy, the Agricultural Advisory Services, and planning and archaeological departments is briefly recorded. There are also two maps showing the areas in which field work has been completed or is in progress.

Pituitary Transplants in the Teleost

J. N. Ball and his colleagues have taken very effective advantage of the development of a technique for hypophysectomizing the viviparous teleost *Poecilia* (*Mollienesia*) *formosa*, and for establishing homografts of the pituitary (*Phil. Trans. Roy. Soc.*, B: *Functional Capacity of Ectopic Pituitary Transplants in the Teleost Poecilia formosa, Comparative Discussion on the Transplanted Pituitary*. By J. N. Ball, Madeleine Olivereau, Anna M. Slicher, and K. D. Kallman. Pp. 69–99+plates 18–21. London: The Royal Society, 1965. 18*s*.; 2.70 dollars). The ectopic gland survives, but without the direct link with the hypothalamus on which its relationships with the central nervous system normally depend. It has thus become possible to analyse with some precision the degree to which the various adenohypophysial functions are influenced by signals from the central nervous system. In some respects the results are similar to those that have been reported from studies of the ectopic pituitary in mammals. Abundant prolactin is secreted by the ectopic graft, but there is a marked reduction or elimination of the secretion of growth hormone and gonadotrophins. But the teleosts have pursued their own independent line of evolution, and it is not surprising, therefore, to find some interesting points of difference in their hypothalamo-adenohypophysial relationships. Corticotrophin secretion seems to be less dependent on hypothalamic stimulation than it is in mammals, and in this respect there is some resemblance between *Poecilia* and amphibians. More unexpected is the finding that the ectopic adenohypophysis of the fish secretes thyrotrophic hormone at a higher rate than normal. This means that in the intact animal the hypothalamus must be exerting an inhibitory influence on this function, whereas in mammals, as is well known, the influence is excitatory. The situation in amphibians in this respect is somewhat obscure, although there is certainly some evidence for an excitatory influence. The authors conclude that at present *Poecilia* seems to be peculiar in the way in which its thyrotrophin secretion is regulated.

New Zealand Geochemical Group

At the University of Otago on August 18, 1965, during the annual conference of the New Zealand Institute of Chemistry, a meeting attended by twenty-one interested persons unanimously approved a motion by Prof. D. S. Coombs, seconded by Mr. A. H. Horn, to form a New Zealand Geochemical Group. Officers elected were Dr. J. Rogers, chairman, Mr. S. H. Wilson, secretary, and Dr. A. Ewart, with power to co-opt. This motion crystallized a recommendation from a meeting chaired by Dr. A. J. Ellis at Lower Hutt during the centenary of the New Zealand Geological Survey. The New Zealand Geochemical Group plans to promote discussion and co-operation in geochemical problems between scientists of different disciplines and backgrounds by a newsletter and symposia at conferences. Liaison is also proposed with geochemical societies overseas. It is hoped to arrange a meeting of the Group during the international symposium on "Volcanology" in New Zealand between November 21 and December 3, which a considerable number of geochemists from overseas will be attending. Anyone interested in the Group is invited to register with Mr. S. H. Wilson, Institute of Nuclear Sciences, Private Bag, Lower Hutt.

Bryozoan Research

Sixteen zoologists and palaeontologists from nine countries were participants in the first international conference on "Post-Palaeozoic Bryozoa" held at the University of Stockholm, Sweden, during May 24–31, 1965, with Ivar Hessland and the Geological Institute as hosts. To assure continuation of frequent and informal exchange of information, ideas, and techniques, the conferees established the International Bryozoology Association, membership in which is open to all workers engaged in research on Bryozoa, Palaeozoic as well as post-Palaeozoic. Plans are being made for a second conference in 1968. The Stockholm conference included four days of informal discussion (led by A. H. Cheetham) of morphology, systematics, biometrics, ecology-palaeoecology, and stratigraphy of post-Palaeozoic Bryozoa and four days of collecting (led by Sten Schager and Ole Berthelsen) of Bryozoa from Senonian-Danian deposits in southern Sweden (Scania) and eastern Denmark (Zealand). Further information about the International Bryozoology Association or the *Proceedings* of the Stockholm conference can be obtained from A. H. Cheetham, Geological Institute, Kungstensgatan 45, Stockholm Va.; or from P. L. Cook, Department of Zoology, British Museum (Natural History), London, S.W.7.

The Official University of the Congo at Elisabethville

The July 1965 issue of Publications of the Official University of the Congo at Elisabethville contains an "Introduction to the Study of Economic Development: General Theories" by A. Cecchella, who describes representative theories of the evolution of economic thought on problems of development (*Publications de l'Université Officielle du Congo à Elisabethville*, 8. *Introduction à l'Etude du Développement Economique les Théories Générales*, by Aldo Cecchella. Pp. 156. Elisabethville: Université Officielle du Congo à Elisabethville, 1965). There are four chapters. The first deals with economic development in the classic thought of J. B. Say, D. Ricardo and R. Malthus, and the second with the Marxist analysis of economic development. The third is directed to the work of J. Schumpeter and the fourth to the Keynesian analysis of modern theories of economic development.

Announcements

Dr. F. M. Lever has been appointed manager of the Research Laboratories of Johnson, Matthey and Co., Ltd. He succeeds Dr. J. C. Chaston, who has retired from the Company. For the past eighteen years Dr. Lever has been head of Chemical and Extraction Metallurgy Research in the Laboratories.

Mr. P. M. J. Woodhead, senior scientific officer at the Fisheries Laboratories of the Ministry of Agriculture, Fisheries and Food, Lowestoft, has been appointed to the position of scientific and administrative officer at the Heron Island Research Station of the Great Barrier Reef Committee in Queensland, by the University of Queensland.

The second edition of the "Biographical Index of Members, Correspondents and Associates of the Royal Academy of Belgium" covers the years 1769–1963, correcting and completing the first edition published in 1948 (*Index Biographique des Membres, Correspondants et Associés de l'Académie Royale de Belgique de 1769 à 1963*. Pp. 299. Bruxelles: Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1964). The entries indicate the field of work of the member; for deceased members, reference is given to obituary notices or other tributes.

The *Annals of the Library Association, 1877 to 1960*, edited on behalf of the Library History Group by W. A. Munford, is a chronological record of the main events affecting the work of the Association since its foundation in 1877 (Pp. 128. London: The Library Association, 1965. 22*s*.; L.A. members, 16*s*. 6*d*.). Mainly of interest to librarians, it includes dates of publication of certain reports, books or pamphlets which may be of wider utility.

A symposium on "Cellular Plastics", the next in the series of Borough Polytechnic symposia on "Plastics and Polymer Technology", will be held at the Borough Polytechnic during November 4–5. Further information can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

A celebration conference, to mark the 200th anniversary of the founding of the Freiberg Mining Academy in East Germany, will be held at the Academy during November 9–14. Further information can be obtained from Mardie Henry, Lex Hornsby and Partners, Ltd., Wellington House, 125 Strand, London, W.C.2.

A one-day conference on "Access to Information", to discuss the implications of a national bibliographical centre and any other ideas which might improve access to information, will be held in the Beveridge Hall, Senate House, University of London, on October 26. Further information can be obtained from the Research Officer, Library Association, Malet Place, London, W.C.1.

A meeting of the Photobiology Group will be held at the Chester Beatty Research Institute, London, on October 27. The programme will include a paper and conversazione on monochromators for photobiology. Further information can be obtained from Dr. J. D. Moreland, Institute of Ophthalmology, Judd Street, London, W.C.1.

The eighteenth annual conference and exhibit on "Engineering in Medicine and Biology", sponsored by the Institute of Electronic and Electrical Engineers and the Instrument Society of America, with participation of the American Society of Mechanical Engineers, will be held in Philadelphia during November 10–12. Further information can be obtained from Dr. H. P. Schwan, University of Pennsylvania, Philadelphia, Pennsylvania.

The third international conference on "Hyperbaric Medicine", sponsored jointly by the Duke University Medical Center and the Committee on Hyperbaric Oxygenation of the National Academy of Sciences—National Research Council, will be held in Durham, North Carolina, during November 17–20. Further information can be obtained from the Secretariat, Box 2928, Duke University Medical Center, Durham, North Carolina.

The annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh, during November 3–5. The programme will include (1) special sessions on metals, ceramics, structures, crystal physics (lattice dynamics and crystalline imperfections) and instrumentation and techniques, and (2) a symposium on "Texture and Preferred Orientation in Polymers". Further information can be obtained from Paul A. Flinn, Carnegie Institute of Technology, Pittsburgh, Pennsylvania 15213.

Erratum. In the communication entitled "Response of Time-dependent Materials to Oscillatory Motion" by Dr. J. Harris, which was published on p. 744 of the August 14, 1965, issue of *Nature*, equation (5) should read as follows:

$$M(t - t') = \int_0^\infty \frac{R(\mathrm{II})}{\mathrm{II}} \exp - \left(\frac{[t - t']}{\mathrm{II}}\, \mathrm{dII}\right) \qquad (5)$$

SPECTROSCOPY IN AUSTRALIA

THE fifth Australian Spectroscopy Conference, sponsored by the Spectroscopy Committee of the Australian Academy of Science and organized by a Committee under the chairmanship of Dr. A. R. H. Cole, was held at Perth, at the University of Western Australia, during May 31–June 2.

The Conference was opened by Dr. A. L. G. Rees, who welcomed those attending of whom fourteen were from overseas. The programme consisted of morning and afternoon sessions on each of the three days, usually with concurrent lectures on topics in different sections of spectroscopy The sections were infra-red and Raman spectroscopy; visible, ultra-violet and theoretical spectroscopy, nuclear and electron spin resonance spectroscopy; atomic absorption spectroscopy; mass spectroscopy and X-ray fluorescence and electron absorption spectroscopy. More than eighty-five papers were presented, of which nine were review papers by invited speakers.

The section on infra-red and Raman spectroscopy opened with a review paper given by Prof. N. S. Sheppard (University of East Anglia) on infra-red band shapes and rotational motion in condensed phases. It was shown that simple molecules such as methyl chloride undergo at least hindered rotation in solution while molecules adsorbed on to surfaces may rotate about the bond linking the molecule to the surface.

The first of the succeeding papers of this section was a report by A. D. E. Pullin (Monash University) on the operation of a commercially made interferometer designed to measure infra-red spectra down to 20 cm^{-1}. A brief outline of the theory of operation and the factors determining spectral range and resolution was given. Examples of spectra obtained were shown.

Several succeeding papers discussed the use of infra-red and Raman spectra to assign particular molecules to their correct point group or to indicate preferred conformations of flexible molecules. J. F. Horwood (C.S.I.R.O., Division of Dairy Research, Melbourne) and J. K. Wilmshurst (C.S.I.R.O., Division of Chemical Physics, Melbourne) suggested that dimethyl oxalate in the liquid and vapour phases was non-planar with point group symmetry C_2. E. Spinner (Australian National University) reported Raman polarization data for aqueous solutions of formates and pointed out that these were consistent with C_s rather than C_{2v} symmetry for the formate ion. N. S. Ham (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the spectra of thionyl aniline, C_6H_5NSO, and showed that it was consistent with a *cis* planar CNSO structure though the *trans* structure could not be eliminated. R. A. Cummins (Defence Standards Laboratories, Melbourne) discussed the preferred conformations present in alkyl tin compounds of the type R_nSnCl_{4-n}, and H. Lee and J. K. Wilmshurst (C.S.I.R.O., Division of Chemical Physics, Melbourne) the conformations of trimethyl orthoformate and tetramethyl orthocarbonate. The vibrational data are consistent with an unsymmetrical *trans-gauche–gauche* arrangement of the methyl carbons with respect to the formate hydrogen in trimethyl orthoformate and with a distorted S_4 structure for the tetramethyl carbonate.

In a second paper concerned with the infra-red spectra of several acetates (hydroxyanthraquinone acetate, phenyl acetate, α and β naphthyl acetates) these authors attribute the observed doubling of the carbonyl stretching absorption to Fermi resonance with combination tones. A. R. H. Cole and L. M. Engelhardt (University of Western Australia) analysed the rotational fine structure of several bands of acrolein in terms of an approximate symmetric top and showed that the data are consistent with the existence of only the *trans* form. Analyses of vibration–rotation bands of methylphosphine and isotopic derivatives, which are also approximate symmetric tops, leading to values of $\frac{1}{2}(B + C)$ in agreement with microwave data were reported by A. G. Moritz (Defence Standards Laboratories, Melbourne). In a later paper A. G. Moritz discussed the vibrational assignment of CH_3SCN and related molecules. G. C. Barraclough and M. Sinclair (University of Melbourne) discussed the vibrational assignment of dicarbonyldinitrosyl iron, and D. E. Clegg (University of Queensland) that of $(CH_3)_3Pt(NH_3)_3^+$ ($(CH_3)_3Pt(NH_3)_3Cl$ in water and deuterium oxide). M. S. Farrell and T. L. Whateley (A.A.E.C. Research Establishment, Lucas Heights) reported the infra-red spectra of beryllium nitrate complexes with organo-phosphorus compounds such as tri-*n*-butyl phosphate, tri-*n*-octyl phosphine oxide and di-(2-ethylhexyl)-phosphoric acid. B. H. James and P. E. Rogasch (Weapons Research Establishment, Adelaide) discussed the changes of the in-plane bending frequencies of substituted phenols with respect to electronic effects and hydrogen bond formation, and R. H. Laby (Department of Agriculture, Melbourne) and T. C. Morton (School of Biochemistry, University of Melbourne) discussed inter- and intra-molecular hydrogen bonding in hydroxy- and nitro-hydroxy-coumarins.

Three papers on the use of infra-red spectroscopy in adsorption studies were presented: M. Clark, B. A. Morrow, N. Sheppard and J. W. Ward (University of Cambridge and the University of East Anglia) discussed the infra-red spectra obtained from the examination of the systems olefine–silica supported nickel (or supported platinum)–hydrogen; N. W. Cant and L. H. Little (University of Western Australia) the spectra for ammonia and deutero ammonia adsorbed on silica; and W. I. Stuart and T. L. Whateley (A.A.E.C. Research Establishment, Lucas Heights, N.S.W.) the spectra of carbon monoxide, of carbon dioxide, and of water on magnesium oxide and on beryllium oxide. Below 200° C carbon dioxide is chemisorbed on beryllium oxide, to form a carbonate and also a singly charged anion, probably CO_3. Above 300° C only the carbonate is formed.

Several infra-red investigations of compounds of biological interest were described. A. J. Michell described polarized infra-red studies of some monosaccharides, related to cellulose and xylan, which were chosen because their crystal structures had been investigated and because they were more easily crystallizable than higher members of the sugar series. E. G. Bendit (C.S.I.R.O., Division of Textile Physics, Sydney) discussed the changes of the infra-red spectra of α- and β-keratin of horse-tail hair on hydration and deuteration. Deuteration tests on α-keratin showed that helical peptide NH groups begin to deuterate immediately, while non-helical peptide NH groups continue to exchange throughout the process. R. D. B. Fraser and E. Suzuki (C.S.I.R.O., Division of Protein Chemistry, Melbourne) considered the roles of inter- and intra-molecular hydrogen bonds in modifying the characteristic vibrations of polypeptides. In an interesting paper R. H. Laby (Department of Agriculture, Melbourne) discussed the infra-red spectra of glycine, glycylglycine and β-alanine in aqueous salt solutions and related spectral changes to conformational changes brought out by differing salt solutions.

Two interesting papers in this section were contributed by visiting Indian scientists. Prof. R. S. Krishnan (Indian Institute of Science, Bangalore) gave an account of the theory of Raman spectra of cubic diatomic crystals; and Prof. C. N. R. Rao (Indian Institute of Technology, Kanpur), in a paper on charge transfer and hydrogen-bonded complexes of *n*-donors, surveyed correlations of spectroscopic and thermodynamic data with substituent effects and other structural parameters. Also concerned with complexes of the charge-transfer type was a paper by

N. F. Cheetham and A. D. E. Pullin (Monash University), who described an infra-red study of molecular complexes between perfluoroalkyl bromides (and iodides) or perfluoroaryl bromides (or iodides) and tertiary amines. The stoichiometry of the complexes had been previously determined by phase studies.

Of wide interest was a paper by G. R. Hunt and J. W. Salisbury (Lunar and Planetary Research Station, Bedford, Massachusetts) on the infra-red techniques used in an attempt to gain information about the silicates present in selected areas of the Moon's surface. These techniques were based on selective reflexion of the radiation by silicates of the various chemical types that might be present on the Moon's surface. A standard spectrometer, suitably modified, was used in conjunction with 42-in. and 69-in. reflecting telescopes.

Many of the papers presented in section *B*, that on visible, ultra-violet and theoretical spectroscopy, reflected the present-day strong interest in problems peculiar to transitions in condensed phases on one hand, and on the other to the new problems that obtrude when the spectra of larger molecules are examined in the vapour phase by high resolution methods.

Prof. D. P. Craig (University College, London), in an invited paper, reviewed the recent advances in the theory of molecular excitons. Prof. Craig described first the modifications of the theory of crystal spectra consequent on taking into account the finite time of propagation of electrical fields in solids, and then discussed, among other topics, how application of theory to crystal spectra could give information on long-range interactions between molecules. Prof. Craig ended his lecture by showing how information about the degree of order in melts could be obtained from their electronic spectra, taking azulene as his example. In a subsequent paper R. G. Body (University of Sydney) gave the results of a mathematical investigation, based on the principles of coupled oscillators, of the positions and polarization of absorptions in crystals due to impurities. Approximate formulae derived enabled the normalized second moment of the energy-level distribution in the pure crystal to be deduced from the spectra of isotopic impurities. Under certain conditions non-isotopic impurities can display striking intensity anomalies. The theme of solid-state spectra was also the subject of papers by G. W. Robinson (California Institute of Technology) on exciton structure in benzene, who gave experimental data on the exciton splitting in the benzene $^1B_{2\mu}$ electronic state and in a number of vibrational levels in the ground electronic state, and by M. Chowdhury, D. McClure and E. Zalewski (University of Chicago), who described an investigation of the Zeeman effect on the singlet triplet spectrum of pyrazine. Single crystals at $\sim 4^\circ$ K and pulsed magnetic fields up to 200,000 gauss were used. Two bands, believed to be two Davydov components, at 26,254 and 26,261 cm^{-1} were assigned, from their polarization and Zeeman effect characteristics, to an electric dipole transition along the N—N axis and to a magnetic dipole transition respectively. Later in the Conference, in an invited address, Prof. McClure described the way in which spectroscopic states of various ions are split when they are subjected to crystal fields of cubic lower symmetry. He illustrated this for various transition elements and rare earth ions, and showed how some complex crystal spectra yielded to this form of analysis. B. N. Figgis and L. G. B. Wadley presented preliminary polarization data on a charge transfer band at about 24,000 cm^{-1} in dilute solid solutions of potassium ferricyanide in potassium cobalt cyanide. Several papers concerned with solvent effects on electronic bands were presented. N. S. Bayliss and G. Wills-Johnson (University of Western Australia) examined the effect of solvents on the intensities of electronic bands of molecules in solution by perturbation methods, deriving the perturbation from the solvent reaction field. The shortcomings of, and possible improvements to, this approach were outlined. The predictions of the theory were compared with experimental results for the $n \rightarrow \pi^*$ transitions in aliphatic ketones and nitroparaffins in solution. D. W. James (University of Queensland) and C. R. Boston and G. P. Smith (Oak Ridge National Laboratory, U.S.A.) described the effects of change of temperature and change of cationic species on the weak band at ~ 195 mμ and the strong band at ~ 300 mμ in molten nitrates in terms of coulomb interactions, variations in environmental symmetry and variation in vibrational energy.

The third invited speaker was Dr. D. A. Ramsay (National Research Council, Ottawa). Dr. Ramsay began his address by pointing out that in general, in electronic spectra, doppler motion broadens lines sufficiently to limit useful resolution to one in half a million, a natural limit attained by the best present-day spectrographs. For larger molecules, the lines of which are closer than this, the observed band contour can be compared with contours computed from overlapping line intensities. (Later in the conference the application of this procedure to part of the azulene spectrum was described by A. J. McHugh of the University of Sydney.) Dr. Ramsay then turned his attention to spectra of smaller molecules recently obtained at the Ottawa laboratories. The structures of glyoxal in the ground and excited states were being deduced from its spectra. Preliminary estimates of bond-lengths for the first excited singlet state were in agreement with predictions of molecular orbital theory for conjugated molecules of this type (glyoxal, butadiene, etc.). After reference to recently obtained spectra of the allyl and glyoxal radicals, Dr. Ramsay described techniques being developed at the Ottawa laboratories to aid the analyses of spectra. The '*J* sorting' technique was based on the injection of microwave power to disturb the rotational levels of one of the combining states. This technique was limited to molecules having a microwave spectrum. In a later paper with Prof. A. D. Buckingham (University of Bristol), Dr. Ramsay described experiments carried out at the Ottawa laboratories on the use of Stark modulation to help identify the type of transitions. An alternating voltage was applied to the plates of a Stark cell at 400 c/s and the Stark-modulated spectrum recorded at high resolution with the photomultiplier detector connected to a lock in amplifier tuned to 800 c/s so that only lines with appreciable Stark effect were recorded. Up to 80 kV was applied across the 3-cm width of the 2-m long cell. A relatively high pressure (~ 300 cm) of SF_6 was introduced in the cell to prevent electrical breakdown. For a symmetric top the Spark splittings are dependent on $\mu KM/J(J+1)$ so that Stark modulation favours the selection of lines of higher K and lower J values. By use of polarized light the Q lines can be identified as those more prominent in parallel polarization and those more prominent in perpendicular polarization as P and R lines. As well as helping to assign lines as P, Q or R, dipole moments of molecules in excited states could be obtained from Stark splittings.

In the field of vapour phase aromatic spectra G. C. Morris (University of Melbourne) presented results on band positions, oscillator strengths and assigned transitions for the region 20,000–54,000 cm^{-1} of the absorption spectra of naphthacene. J. P. Byrne and I. G. Ross (University of Sydney) considered the cause of spectral diffuseness: not all diffuseness is due to predissociation; plain congestion of the spectrum can be a cause in larger molecules. L. M. Logan and I. G. Ross (University of Sydney), in a paper concerning molecular fluorescence in the vapour state, considered the interaction between the original excited vapour state and the vibronic states of the lower electronic levels. R. H. Kennet (Weapons Research Establishment, Adelaide) described a method for the determination of the intensity distribution in an unresolved molecular band, and described features of the profile that are dependent only on temperature and from which the temperature may be deduced.

In an interesting paper C. E. Kendall (National Biological Standards Laboratory, Canberra) attempted to delineate more closely the possible connexion between the carcinogenic activity of molecules and their electron donor characteristics by using experimental data and energies of the highest occupied molecular orbitals of the donors to prepare a common index of donor activity for comparison with charge transfer data. When this comparison is made—for example, in the form of a plot—complexes of nearly all carcinogens lie in a fairly narrow range, especially when the common acceptor is chloranil. Possibly some type of electron transfer may be involved in a cell, with an electron acceptor of similar affinity to chloranil. B. K. Selinger (School of General Studies, Canberra) in a paper on excimer formation described an investigation of the effect of pressure on the kinetics of formation of excited dimers by molecules in the excited state. Where the activation energy of dimer formation is low, the increase in viscosity with pressure does not affect the process and the decrease in volume accompanying excited dimer formation can be measured.

The diversity of uses of spectroscopic techniques was exemplified by a paper by K. J. Taylor and P. D. Jarman (University of Western Australia) on an aspect of sonoluminescent spectra. When cavities in liquids, produced ultrasonically, collapse, thermally excited radiation is emitted. If the liquid contains a dissolved alkali metal salt, the alkali lines appear in emission displaced and much broadened by interactions with the cavity contents. From these data the relative density of the cavity contents at the time of light emission can be calculated.

Instrumental aspects of spectroscopy were represented by several papers. Dr. S. E. Williams (University of Western Australia), in a review of the development of spectroscopy in the spectral range 1000–20 Å, described the many experimental problems encountered and showed examples of spectra in this region. Dr. Williams emphasized the importance of isoelectronic sequences in the interpretation of such spectra. S. C. Baker (University of Newcastle) described an Ebert-type scanning spectrometer with a resolving power of 500,000 in the tenth order green, and K. J. Taylor and P. D. Jarman described an auto-spectrophotometer for a low-intensity source. The prism can be rotated in either direction in steps by a motor driven by electric pulses derived from a logic circuit which also addresses the integrated output pulses from the photomultiplier into a 400-channel analyser. The memory of the analyser can then be read out as desired.

In a paper on the electronic emission spectrum of the diatomic molecule IF, R. A. Durie (C.S.I.R.O., Division of Coal Research, Sydney) summarized the analysis of the $A'\Sigma \rightarrow X^3_{\pi 0}$ transition and the derived molecular constants. C. Candler (Bendigo Technical College, Bendigo) presented a paper on the mechanism of dye sensitization of photographic emulsions.

Two other invited review papers related to electronic spectroscopy were given during the conference. Dr. Wilse Robinson (California Institute of Technology) gave a stimulating account of photosynthesis from a spectroscopist's point of view, stressing the essentials and the two-quanta process involved; and Prof. R. D. Brown (Monash University) outlined the best methods now available for calculating the energies of excited states of molecules. Introduction of configuration interaction led to a large number of integrals. Their number could be reduced by the approximation of zero differential overlap. Improved energies could be obtained by semi-empirical corrections to decrease nearest neighbour repulsion integrals to allow for the effects of electron correlation. Prof. Brown ended his address by showing how the calculated energies of the electronic states of benzene varied according to which of the up-to-date methods of correcting these integrals was adopted.

Two papers in this section formed a close link with those in the mass spectroscopy section. J. D. Morrison (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the application of extended Hückel type calculations on simple molecules to determine if possible the location from which an electron will be ionized and, if the resultant ion dissociated, the most probable fragments and their structures, and A. J. C. Nicholson (C.S.I.R.O., Division of Chemical Physics, Melbourne) reported an investigation of photo-ionization efficiency curves. Ionization potentials of methane, ethane and propane, claimed to be closer to the adiabatic values than those extant, were reported, and it was shown how, from a comparison of photo-ionization efficiency curves, it is possible to identify vibrational and excited states of specific molecular ions.

Section *C*, dealing with nuclear and electron spin resonance spectroscopy, covered a fairly wide range of interests despite the small number of papers presented.

The analysis of high-resolution nuclear magnetic resonance spectra was considered in a paper by S. N. Stuart (C.S.I.R.O., Division of Chemical Physics, Melbourne), who showed how it was possible to systematize the use of quantum mechanical trace relations in such analyses and illustrated some of the simplifications thus arising.

In the field of proton resonance spectroscopy, the paper by P. J. Black, R. D. Brown and M. L. Heffernan (Monash University) showed how it was possible, after appropriate corrections had been applied, to correlate proton shifts with molecular orbital electron densities of aromatic heterocycles. R. G. Gillis (Defence Standards Laboratories, Victoria) and N. S. Ham (C.S.I.R.O., Division of Chemical Physics, Melbourne), in studies on a number of methyl–sulphur compounds, used the relation between proton chemical shifts and ^{13}C-H coupling constants to estimate the anisotropy effects present in these substances. The effectiveness of natural abundance ^{13}C-nuclear magnetic resonance spectroscopy as a tool for the analysis of coal derivatives was well illustrated in the paper by R. A. Friedel and H. L. Retcofsky (Bureau of Mines, Pittsburgh Coal Research Centre).

Broad line NMR was well represented by three papers. C. K. Coogan (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the investigation of charge distributions in the ionic crystals LiOH and NaSH and showed how a number of other crystal properties could be investigated with NMR. A similar investigation of ionic crystal with a linear triatomic anion was reported by I. D. Campbell (C.S.I.R.O., Division of Chemical Physics, Melbourne). G. J. Jenks (Defence Standards Laboratories, Melbourne) demonstrated the use of tin and nitrogen resonances in the assignment of a structure to tri-*n*-butyltin thiocyanate and reported the presence of an ^{119}Sn–^{15}N coupling constant.

Electron spin resonance spectroscopy was represented by one paper. K. Kenny (Washington University, St. Louis, U.S.A.) indicated how the use of multi-channel analyses has facilitated the detection of transient free radicals. The application of the technique to living aquarium plants was described.

The section on atomic absorption revealed exciting developments in all aspects of the field. In his invited contribution, A. Walsh (C.S.I.R.O., Division of Chemical Physics, Melbourne) described the successful development by him and his colleagues of several new systems for enhancing and isolating resonance radiation. These include a hollow cathode lamp having a subsidiary discharge that selectively intensified resonance lines 100-fold without increasing line width, and resonance monochromators that can be used both with conventional and high-intensity lamps and that offer the possibility of simultaneous determination of a number of elements. A. Hell (Beckman Instruments, U.S.A.) discussed the optical and mechanical factors involved in obtaining optimum performance from a laminar flow burner and described means of extending analytical range by the use of an infra-red heated spray chamber and solvent conden-

sation system. The addition of 12 elements to those previously determinable in aqueous solution and a general alleviation of chemical interference by the use of high-temperature flames employing O_2–N_2 mixtures and N_2O with acetylene was revealed by J. B. Willis (C.S.I.R.O., Division of Chemical Physics, Melbourne) in a joint paper with M. D. Amos (Techtron, Victoria). In another paper, J. B. Willis stated that examination of atomizer design has resulted in considerable improvement in performance being achieved by precision in construction and by providing means of accurately positioning the capillary. J. V. Sullivan (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the construction and performance of high-intensity lamps. The increase in the intensity of the resonance lines has been achieved by exciting the metal atoms, produced by a hollow-cathode discharge with a secondary discharge electrically isolated from the former. He then described a high-intensity lamp which incorporates an additional hollow cathode which selectively modulates the resonance lines and permits the use of low-resolution monochromators for the isolation of resonance lines in complex spectra. W. Slavin (Perkin-Elmer, U.S.A.), in tracing the development of burners, described a design having multiple slots for producing an N_2O—C_2H_2 flame of high temperature and stability by the operation of sheathing flames on each side. Examination of the performance of this burner has shown that good agreement with other methods is obtained, that a number of transitions from excited states absorb and that phosphate enhances vanadium absorption. Mrs. J. A. Bowman (C.S.I.R.O., Division of Chemical Physics, Melbourne), in collaboration with J. C. Heerdt (Commonwealth Railways, Port Augusta, South Australia) and J. B. Willis, reported that good agreement could be obtained between atomic absorption measurement on oil dissolved in 2-methyl-4-pentanone and both atomic absorption and colorimetric results on dissolved ash for Cu, Cr, Fe, Pb and Ag, but it was agreed in discussion that the greatest hazard in diagnosis of engine breakdown lies in the sampling of the oil. N. J. Marshall (Bureau of Mineral Resources, Canberra) reported success in the extraction of various substituted thiocarbamate complexes of Cu, Pb, Ni, Co and Te into amyl acetate for the determination of these elements, at parts-per-hundred-million levels, in rocks by atomic absorption; and D. C. Bowditch, jointly with J. A. Powell (both of the Australian Mineral Development Laboratories, Adelaide), that atomic absorption could compete favourably with other means of analysis in the determination of Cu, Zn, Co, Ni, Cd, Mn, Sb, Ag and Bi both at low concentration in geochemical and mill tailing samples and at high concentration in mill feed and product control. After outlining his methods of soil and plant analysis for some 25 major and minor elements by means of flame emission, porous cup spark emission, rotating disk spark emission on a direct reader and chemical concentration followed by cathode layer arc emission (improved by image converter scanning of plates), R. L. Mitchell (Macaulay Institute for Soil Research, Scotland) described, in an invited address, an atomic absorption method for the determination of extractable cobalt at deficiency-levels in soils, stating that considerable gain in sensitivity had recently been achieved by use of a special atomizing system, tuned a.c. amplifier and recorder read-out.

A successful mass spectrometry section was a late addition to the Conference programme. Seven papers were presented; six of these, including an introductory lecture by J. S. Shannon (C.S.I.R.O., Division of Coal Research, Sydney), dealt with structural applications in organic, metal-organic and co-ordination chemistry, and the seventh with an electric quadrupole mass spectrometer (D. Swingler, C.S.I.R.O., Division of Chemical Physics, Melbourne). The structural applications reported included a structural assignment for the complex fungal metabolite sporidesmin *C*, a tentative structure for the cell growth factor 'Zeatin', a characteristic reaction of 2,5-diaryl oxazole systems in which CO and HCN is eliminated (W. D. Crow, J. Hodgkin, R. Hodges, D. Letham and J. S. Shannon), structures for the Schiff Base *bis*benzoylacetone-ethylenediimine and its metal chelates, and the mass spectra of Ph_3M (M = N, P, As, Sb, Bi) and related compounds (J. S. Shannon) and of macrorungia alkaloids (R. R. Arndt, K. Biemann, A. Jordaan, V. P. Joynt and J. L. Occolowitz (C.S.I.R.O., Division of Chemical Physics, Melbourne). N. Wasada and I. Tsuchiya (Government Chemical Industrial Research Institute, Tokyo), E. Yoshii (Faculty of Pharmaceutical Sciences, University of Toyama, Japan), T. Fukuzumi (Japan Monopoly Corporation) and E. Watanabe (Japan Electron Optics Laboratory) reported on fragmentation patterns and discussed their mechanisms for santonins and their derivatives and also for solanone, an unsaturated branched aliphatic ketone from tobacco leaf. J. L. Occolowitz (Defence Research Laboratories, Melbourne) and J. M. Swan (C.S.I.R.O., Division of Organic Chemistry, Melbourne) described the mass spectra of phosphonic esters $R.PO(OR')_2$ and dichlorides $RPOCl_2$, and emphasized the value of keeping an accurate electron balance in the formulation of ionic equations. General patterns for the decomposition of odd electron (radical) ions and even electron ions were described. In a stimulating discussion of these papers the approach of the organic chemists to the interpretation of mass spectra was criticized as lacking quantitative energy factors; in reply it was maintained that, provided the approach was utilized correctly, there was at present no better method available.

The remaining section of the Conference consisted of four papers on X-ray spectroscopy. D. McKenzie (Australian Iron and Steel, Wollongong, N.S.W.) described the analysis of coals by X-ray spectroscopy, and in a paper by K. Norrish (C.S.I.R.O., Division of Soils, Adelaide) a technique for silicate analysis was described in which the sample was fused with lithium borate glass, with added lanthanum oxide and cast in a penny-shaped disk. Advantages of this technique include durability of sample and uniform background. K. Norrish and C. G. Gurr (C.S.I.R.O., Division of Soils, Adelaide) described an automatic pulse-height analyser for use in X-ray spectrography, and J. B. Swann (University of Western Australia) the energy of electrons scattered by silver with surface contamination.

The sixth Australian Spectroscopy Conference will be held in Brisbane in 1967.

G. C. Barraclough
P. J. Black
D. J. David
A. D. E. Pullin
J. S. Shannon

BRITISH AID TO DEVELOPING COUNTRIES

A WHITE PAPER from the Ministry of Overseas Development*, outlining the work of the new Ministry, describes the policies by which the British Government proposes to be guided and the ways in which it hopes to help to meet the needs of the developing countries, without, however, specifying the extent of Britain's aid in particular periods. Successive chapters outline motives and objectives, the pace of overseas development, the transfer of resources and Britain's capacity to help—in

* Ministry of Overseas Development. *Overseas Development: The Work of the New Ministry.* (Cmnd. 2736.) Pp. 74. (London: H.M.S.O., 1965.) 5*s.* 6*d.* net.

which the Department of Technical Co-operation had laid firm foundations. However, it does not really meet the criticism which has come from the Overseas Development Institute, or from the Estimates Committee in its tenth report for the session 1963–64. Mr. R. Carr, then head of the Department of Technical Co-operation, had indicated that Britain's contribution, £190 millions in 1964, would rise to about £220 millions, and since the White Paper was published it is understood that this figure will probably be increased to £250 millions.

The White Paper makes scarcely any reference to the application of science and particularly of research, and this weakness, exemplified notably in the disbandment of the Overseas Research Council, therefore persists. The initiatives in technical assistance which are being taken in the economic services seem a little inadequate. Certainly, it is unfortunate that in a statement on the White Paper in the House of Commons on August 3 the Minister should have been so petulant and inclined to make a party issue of a matter which has the support of all parties and for which Mr. Carr had laid such good foundations. Mrs. Castle said that the levels of expenditure on aid would be contained in the national plan. It was proposed to establish a careers service in the Ministry for a corps of specialists directly employed by the Ministry who would normally be on loan overseas. The Ministry was also setting up an Overseas Service Pensions Fund to provide pension rights for persons serving overseas for which such provision was not at present made.

Much in the White Paper supplements an earlier statement from the Department of Technical Co-operation in 1963, but further chapters deal with the lines of future policy, the new terms of aid, the private sector and the Commonwealth Development Corporation, the management of aid, and with what are described as "new initiatives in technical assistance", including recruitment and voluntary service, economic services, education, training and public health. It is emphasized that the Ministry would like to see it widely accepted in Britain that a professional career should normally include a period of work overseas in a developing country. The Government has decided, therefore, that in fields of recruitment where it is important to ensure that British help is available, the home establishments of Government departments and public bodies should be strengthened so as to make it possible to release people more readily. Discussions with other Government departments and the University Grants Committee have shown that there is scope for strengthening certain establishments and that the organizations concerned are willing to co-operate. A total of at least 400 posts will be added for this purpose to home establishments, in Government departments, universities, technical colleges, etc.

On service overseas by volunteers, the White Paper, noting that the number of such volunteers is expected to rise from 1,400 in the autumn of 1965, to 1,800 in 1966, points out that it is important to widen the range of work which volunteers are doing and to draw them from wider sections of our society. On the economic services, more economists and statisticians are being supplied for periods of service overseas, directly attached to Governments; it is hoped to increase this number by the recruitment measures just described. A survey of British universities is being undertaken to learn more about the number and type of economists interested in, and available for, work overseas; the supply of such people will be increased by a new Institute of Development Studies in accordance with the recommendation of the Bridges Committee in 1962. Up to 1,000 non-university teachers are to be recruited in 1965 and almost £200 millions a year has been spent on the supply of teachers under the Overseas Service Aid Scheme alone. The provisions of the Overseas and Development Service Bill will help to increase new recruitment and retain the services overseas of British staff employed other than by Governments. The Commonwealth Teacher Training Bursary Scheme has been stepped up to 500 and will be increased to 550 next year. The number of students and trainees coming to Britain under the Ministry's programme has risen from just more than 2,000 in 1962–63 to nearly 2,800 in 1964–65.

Under the terms of aid, the White Paper refers to the decision to make development loans free of interest where appropriate. It is claimed that the new structure under the Ministry of Overseas Development has strengthened Britain's work on aid in various ways and, after detailing the extent of British aid, there is a brief appendix on the organization of the Ministry. The objective of Britain's aid programme is to help developing countries in their efforts to raise standards of living. Its basis is accordingly a moral one, but it is recognized that provision of aid is to Britain's own long-term economic advantage and that she gives aid because, in the widest sense, it is believed to be in her interests to do so as a member of the world community. British or any other aid is most effective where it forms an integral part of a coherent and co-operative effort to implement a well-prepared development plan.

The White Paper is well written and cogently argued. It directs attention to such obstacles to the development of the poorer countries as the shortage of professional and technical personnel, which in many countries had been even more serious than shortage of foreign exchange in inhibiting development. It also notes the difficulties caused by the accelerated growth of population and, as a consequence of economic and demographic trends, a general failure to find productive employment for the labour force. Its silence in regard to scientific research is its outstanding defect, and from this it is apparent how vulnerable some of the proposals are to the incidence of the Government's new measures of restricted expenditure.

SOLAR MOTION AND SUNSPOT COMPARISON

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SEVERAL attempts have been made to associate the position of the planets with the 11·1-year cycle of solar activity[1]. This article presents some preliminary results of a solar dynamic investigation based on classical dynamics. The inertial frame for bodies in the solar system is the centre of mass of the solar system, not the centre of the Sun[2]. It is known that the centre of the Sun can be greater than one solar radius distant from the solar system centre of mass if Jupiter and Saturn are in the same general direction in space[3]. This motion of the Sun with respect to the solar system centre of mass will lead to forces on and in the Sun which vary with time and heliographic position. Although the spatial relationships between the Sun and the solar system centre of mass

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have been examined and reported, we are aware of no systematic investigation of the attendant velocity, acceleration, or jerk, an accepted term for rate of change of acceleration.

To accomplish such an investigation, the appropriate three-dimensional vector operations were coded for use with the IBM 7094 computer, using an autogenerating ephemerides sub-routine as an input. For example, the position, velocity, acceleration, and jerk of the Sun are given, respectively, by:

$$\vec{r}_{cm1} = -\sum_{i=2}^{10}(m_i)\,({}_1\vec{r}_i)/\sum_{i=1}^{10} m_i$$

$$\dot{\vec{r}}_{cm1} = -\sum_{i=2}^{10}(m_i)\,({}_1\dot{\vec{r}}_i)/\sum_{i=1}^{10} m_i$$

$$\vec{a}_{cm1} = -G\sum_{i=2}^{10}(m_i)\,({}_1\vec{r}_i)/\,|{}_1\vec{r}_i|^{3}$$

$$\dot{\vec{a}}_{cm1} = -G\sum_{i=2}^{10} m_i\left(\frac{{}_1\dot{\vec{r}}_i}{|{}_1\vec{r}_i|^{3}} - \frac{3({}_1\dot{r}_i)({}_1\vec{r}_i)}{|{}_1\vec{r}_i|^{4}}\right)$$

where ${}_1\vec{r}_i$ and ${}_1\dot{\vec{r}}_i$ are outputs of the autogenerating ephemerides sub-routine, a pre-subscript refers to the co-ordinate system, and a post-subscript refers to the body in question ($i = 1$ for Sun, $i = 2$ for Mercury, etc.). The body mass is given by m, position by $\vec{\mathbf{r}}$, and acceleration by $\vec{\mathbf{a}}$, and G is the universal constant of gravitation. Pluto ($i = 10$) was omitted because its influence is negligible for the calculations performed.

The four large outer planets (Jupiter, Saturn, Uranus, Neptune) are the most important in determining the position of the Sun[4], whereas the three closest inner planets (Mercury, Venus, Earth) are the most important in determining the jerk of the Sun. Table 1 illustrates this point by summarizing the ranges of the magnitudes of these dynamic quantities. It is particularly noteworthy that the fluctuation of the acceleration due to all the planets is approximately twice that due to the outer planets only; furthermore, the inner planets predominate in their influence on the jerk. Although it is not known whether the jerk itself is a physically important quantity, it represents a useful function which is related to rate of change of force.

Table 1. Ranges of Solar Dynamic Quantities

Planets	Position (10^8 m)	Velocity (m/sec)	Acceleration (10^{-7} m/sec^2)	Jerk (10^{-14} m/sec^3)
All	0–15	9–16	1·4–2·9	0–2·8
Outer	0–15	9–16	1·7–2·5	0·3–0·4
Inner	0–0·01	0·01–0·2	0–0·6	0–2·4

These ranges were obtained by the examination of 100 years of output, most of it at 8-day intervals. IBM 7094 running time is about 1 min per decade. Note the increasing importance of the inner planets with each successively higher time derivative.

The solar acceleration and jerk vectors can be usefully described by three scalars each: magnitude, component along the Sun's axis, and the longitude. The latter is defined as that angle between the planes defined by the zero longitude vector and the Sun's axis of rotation, and the vector of interest and the Sun's axis. The zero longitude vector is essentially fixed in direction in inertial space and lies at the intersection between the plane of the solar equator and the plane of the ecliptic.

The jerk longitude in inertial heliographic co-ordinates is plotted for the beginning of sunspot cycle 17 (1933) and cycle 18 (1944) in Fig. 1.

The detailed similarity of the patterns for the times beginning with 1933 and 1944 does not occur at any intermediate times, although space prevents a display of this point. The jerk along the Sun's axis and the magnitude of the jerk also repeat in a similar fashion, and all these quantities have patterns which repeat with the same period

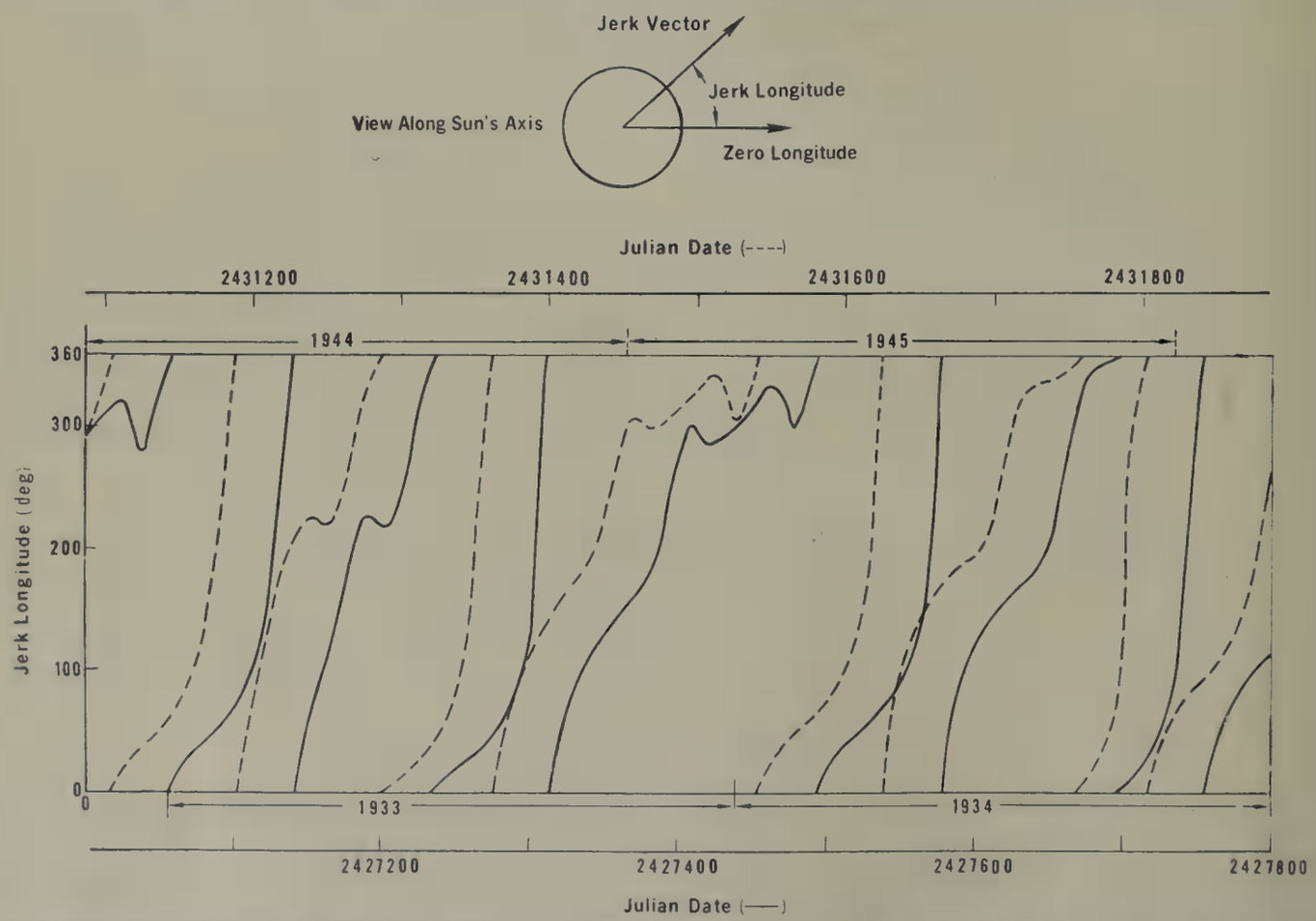

Fig. 1. Jerk longitude during portions of cycle 17 and cycle 18. The longitude is measured in the plane of the solar equator from the ascending node of the intersection between the plane of the solar equator and the ecliptic. The time axes, shown in Julian days, are displaced by 4,085 days. The 1933–34 time axis goes with the solid curves and the 1944–45 time axis with the dashed curves. The pattern separation is determined to be 4,047 days

for cycles 19 and 20. Moreover, although the acceleration has a maximum approximately every 11·86 years (due to Jupiter) the fine structure of the acceleration due to the inner planets has the same repetition period as the jerk. Careful measurements of accurate curves indicate a separation between the patterns of 4,047 days, corresponding to 11·08 years. The average length of the solar sunspot cycle from Waldmeier[5], using maxima from 1610 to 1960, is 11·04 + 0·25 years, and using minima is 11·08 + 0·20 years. This correspondence, while not conclusive, seems unlikely to be accidental.

Fig. 2 shows the comparison between smoothed sunspot Wolf number and the rate of change of jerk longitude. The Wolf number is the daily count of the number of spots plus ten times the number of sunspot groups, and is roughly proportional to the total sunspot area. The similarity of the curves is interesting. The use of rate of change of jerk longitude followed considerable examination of other dynamic function comparisons, namely, acceleration magnitude, jerk magnitude, axial components of jerk and acceleration, and longitude of acceleration. The Wolf number peaks of 1947 were selected to illustrate a case of favourable comparison. Since for other years the similarity of the curves is weaker, this suggests that the rate of change of jerk longitude is not the main variable responsible for an effect on solar activity, if indeed such a causal effect does exist.

There are several ways in which the Sun's motion and attendant accelerations, forces, or their rate of change may trigger solar activity. Three typical ones are: (*a*) small photosphere velocities could result in Coriolis terms leading to cyclones and anticyclones; (*b*) the very unstable convective region several hundred km below the visible surface could be influenced by acceleration, leading to different buoyant forces[6]; (*c*) dynamic effects could push the material associated with strong magnetic fields through the solar surface, as in Babcock's theory[7]. Such mechanisms all seem plausible.

If the motion of the Sun as determined by the gravitational forces of the planets is a causal element for solar activity[4], then many observed facts about solar activity could be explained. The alternation of sunspot polarities with each cycle, the decrease in latitude of activity as the cycle progresses, the multiplicity of cycles[8], the east–west asymmetry of activity, and the observed recurrence of active regions fixed in inertial space all require explanation. By demonstrating a physical link between solar dynamics and solar activity, a more comprehensive understanding of solar physics could be realized. This could ultimately lead to realistic solar activity prediction.

In conclusion, the following points can be clearly stated: (*a*) the influence of the inner planets on the fluctuation of the acceleration of the Sun in inertial space is as important as that of the outer planets; (*b*) the short-time acceleration and jerk patterns are repetitive with a clear 11·08-year period, agreeing well with the mean sunspot cycle.

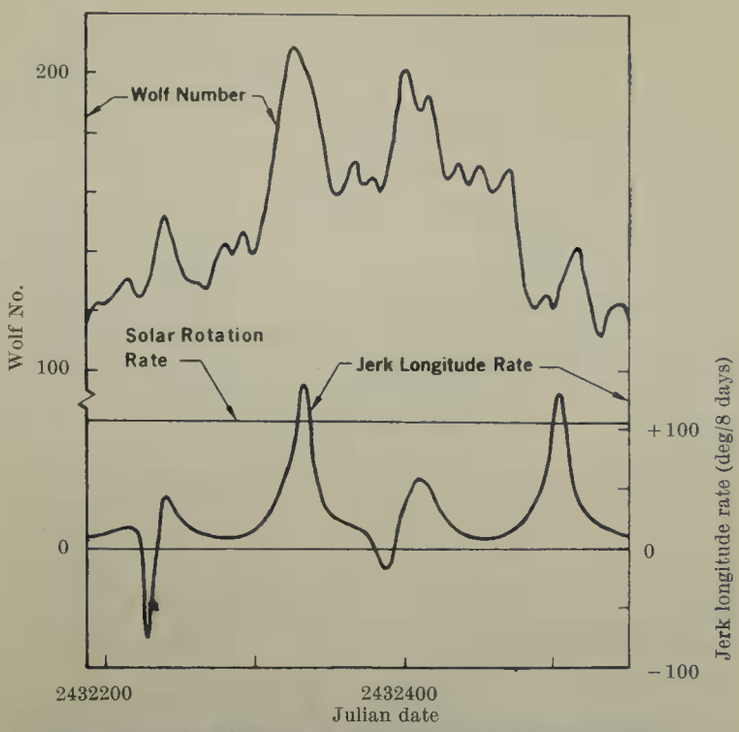

Fig. 2. Comparison between Wolf number and jerk longitude rate for 1947. A 27-day moving average for Wolf number has been used to reduce greatly the effects of solar rotation as seen from the Earth. The rate of change of jerk longitude is the slope of curves like those of Fig. 1. The average solar rotation rate is shown for reference

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STRATOSPHERIC TRANSPORT OF VOLCANIC DUST INFERRED FROM SOLAR RADIATION MEASUREMENTS

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RECENTLY, a number of authors have reported observations of volcanic dust in the southern hemisphere following the eruption of Mount Agung, Bali (8° S., 115° E.), on March 17, 1963. Hogg[1] discussed several optical effects observed at Mount Stromlo and elsewhere. Moreno and Stock[2] measured extinction effects at Cerro Telolo, Chile. Mossop[3] obtained dust samples from aircraft flights at a height of 20 km in latitude belts 15°–35° S. and 40°–45° S. Flowers and Viebrock[4] have reported an anomalous decrease in solar radiation at the South Pole and also at Mauna Loa, Hawaii.

In the northern hemisphere, Meinel and Meinel[5] have estimated the height of the dust layer over Tucson, Arizona, to be 17·8 km in late 1963.

These various accounts contain some features of interest to the meteorologist concerned with atmospheric transport on a global scale. It appears that the volcanic dust moved toward the South Pole in the lower stratosphere at such a rate that the initial detection in middle

latitudes occurred after 5–7 weeks and the maximum concentration appeared after about 4–6 months. These findings bear a remarkable similarity to observations at 38° S. of the arrival of fission debris following the atomic tests in equatorial regions in 1958–62 (refs. 6 and 7).

A very extensive record of solar radiation data has been continuously maintained since 1959 at this laboratory. This has been examined to see if information could be deduced concerning the movement of the dust over a number of years. Similar investigations following the Krakatoa, Mount Pelée, Katmai and other explosions suggested that absorption of the direct solar beam by the dust layer might be observable for several years[8–11].

The data used exist in the form of continuous chart records of the total radiation T and the diffuse radiation D falling on a horizontal surface. These observations are made with Kipp and Zonen pyranometers periodically calibrated against a Linke Feussner pyrheliometer. From the chart records, 'clear' days were selected, that is, days for which there was no evidence of cloud within 1 h of noon, and the maximum values about noon of total and diffuse radiation noted. On the average seven 'clear' days were available for each month with slightly more in summer than in winter.

The direct solar radiation, I, at normal incidence, can be readily calculated from $I = (T - D)/\cos\zeta$ where ζ is the zenith angle. Subsequent analyses were then carried out in terms of two-month averages.

The data for the years 1959–62 were used to establish the norms $\bar{T}$, $\bar{D}$ and $\bar{I}$ for each two-month period. Fig. 1 presents a plot of the quantities $D/\bar{D}$, $I/\bar{I}$, $T/\bar{T}$ and D/T for 1959–65. The time of the Bali eruption is indicated.

It is obvious that some remarkable effects occurred in the radiation levels during 1963 and to a lesser extent in 1964 and 1965. In the light of observations elsewhere it is plausible to associate this with the presence of a layer of volcanic dust from the Bali eruption. The gradual removal of the dust over a period of several years is evident.

The maximum effect occurred during July–August of 1963 when the direct solar radiation was reduced by 24 per cent. A reduction of 16 per cent occurred during July–August of 1964 and there is a suggestion of similar behaviour in 1965.

The most spectacular feature appears in the diffuse radiation record which shows twice normal values during July–August 1963 and continues to show abnormally high values in 1964 and 1965.

The total radiation values show a significant, but less-obvious, reduction in 1963 and 1964. The information contained in Fig. 1, if taken at face value, would indicate that, of the radiation removed from the direct solar beam, more is scattered in a forward than in a backward direction. This would imply only a minor modification of climate as a result of scattering by the dust layer, even when such dramatic effects are evident in the direct and diffuse radiation.

It is clear, however, that the 'signal-to-noise ratio' of the total radiation data in Fig. 1 is not very high. This is caused partly by the presence of pollutants in the urban atmosphere and partly by the use of data from routine observations instead of a specifically oriented experiment. A similar analysis of higher quality data would materially assist the foregoing considerations.

From the point of view of using the volcanic dust as a tracer of atmospheric motions, it is necessary to estimate the amount of dust present at various times.

The change in the direct solar beam dI' along an element at slant path dl is given, in simple scattering theory, by:

$$dI' = -knI'dl - k_0n_0I'dl$$

where k is a scattering coefficient and n is the concentration of dust particles in a thin layer. The term $k_0n_0I'dl$ is the reduction due to scattering by aerosols normally present.

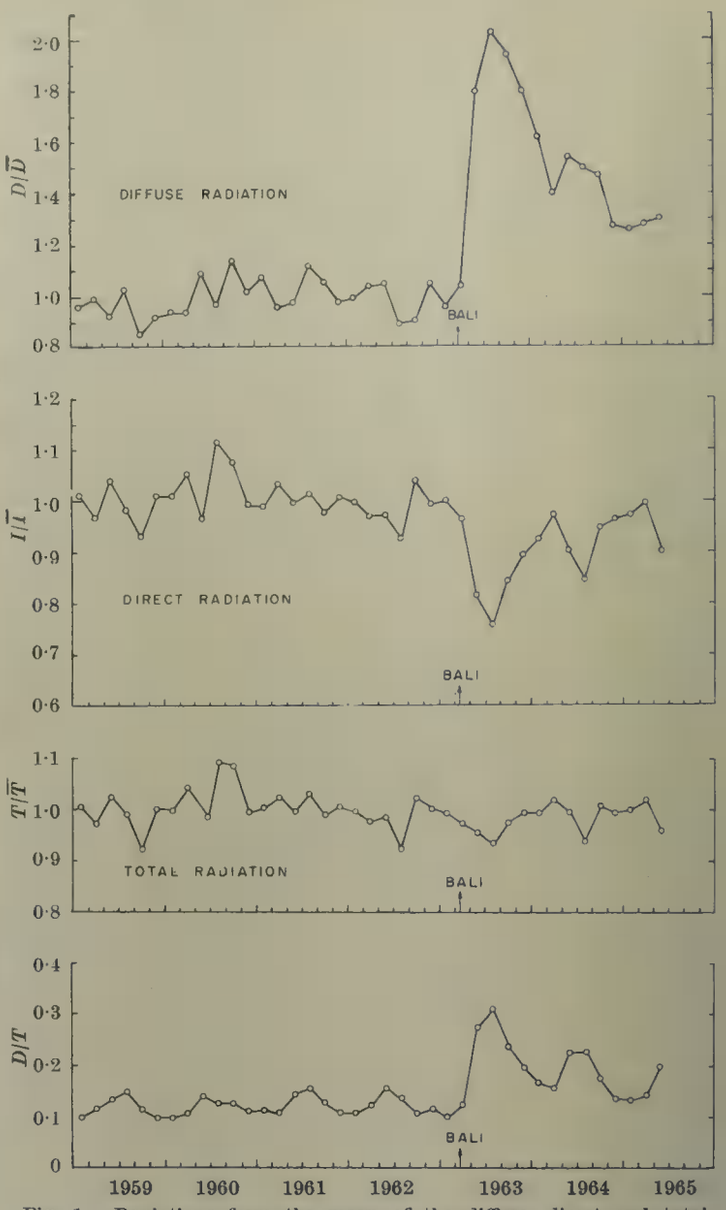

Fig. 1. Deviations from the mean of the diffuse, direct and total solar radiation and the contribution of scattered radiation to the total, 1959–65

Introducing the Earth radius R, the solar zenith angle ζ, the height of the dust layer h and its depth Δh, we have on integration:

$$\ln(I/I_0) = -kn\left\{\sqrt{(R+h)^2 - R^2\sin^2\zeta}\right\}_{h}^{h+\Delta h} - C$$

where I_0 and I are the values of I' outside the atmosphere and at the Earth's surface respectively. C refers to the integral of k_0n_0dl taken throughout the atmosphere. Making the simplifying approximation $R \gg h \gg \Delta h$, we have:

$$\ln(I_0/I) = kn\Delta h/(\cos^2\zeta + 2h/R)^{1/2} + C$$

$\bar{I}$, as previously defined, is the average value of I in the absence of volcanic dust so that $\ln(I_0/\bar{I}) = C$ and hence:

$$\ln(\bar{I}/I) = kn\Delta h/(\cos^2\zeta + 2h/R)^{1/2}$$

Since $n\Delta h$ represents the total number of dust particles in a vertical path we can, by assuming k constant, use the quantity $(\cos^2\zeta + 2h/R)^{1/2}\ln(\bar{I}/I)$ as an index of the amount of dust present at any time. We may, in fact, expect k to change slowly with time owing to the gradual removal of the larger particles. The term $2h/R$ is only important when the Sun is very close to the horizon. At all other zenith angles, $(\cos^2\zeta + 2h/R)^{1/2}$ reduces to $\cos\zeta$.

In Fig. 2 the quantity $(\cos^2\zeta + 2h/R)^{1/2}\ln(\bar{I}/I)$ is plotted from the present data and for the South Pole data of

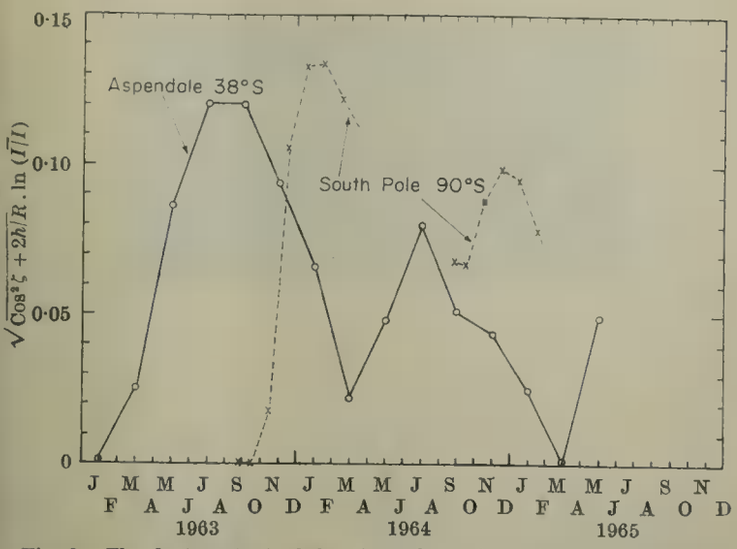
0·15
0·10
0·05
0
Aspendole 38°S
South Pole 90°S
$\sqrt{\overline{\cos^2\zeta + 2h/R}} \cdot \ln(\bar{I}/I)$
J M M J S N J M M J S N J M M J S N
F A J A O D F A J A O D F A J A O D
1963
1964
1965

Fig. 2. The dust content of the atmosphere above Aspendale and the South Pole following the eruption of Mt. Agung on March 17, 1963

Flowers and Viebrock[4,12]. In the latter case, a dust layer altitude of 10 km has been assumed where necessary.

The first major arrival of volcanic dust is seen to occur during July–August of 1963 at 38° S. and during January–February of 1964 at 90° S. At almost the same times in the following year a new influx of dust is apparent at both latitudes, indicating a pronounced seasonal effect in the poleward stratospheric transfer. The existence of an enhanced 'winter' transfer revealed in 1964 would no doubt have contributed to the initial arrival of the volcanic dust in 1963. The indications of Fig. 2 are supported by the occurrence of vivid sunsets over Southern Australia during the winters of 1963 and 1964, and their reappearance at reduced intensity during the present 1965 winter. Similar seasonal effects were observed in 1884 and 1885 following the Krakatoa eruption in 1883 (ref. 8).

Time-lags of similar amounts between temperate and polar latitudes have been observed in studies with other tracers. The maximum in total ozone occurs at 38° S. in September, at 54° S. in October and at 75° S. in December[13]. The maximum in radioactive fall-out in rain occurs over southern Australia during November–December[7]. All these tracers have in common that they originate in equatorial regions. (Ozone is also produced at other latitudes, but in reduced amounts.)

It is clear that stratospheric transfer processes from equator to pole involve times of the order of five months to middle and eleven months to polar latitudes. Whether these take the form of mean motion or eddy diffusion, or some combination of both, cannot be determined from the present studies. However, for the South Pole data Flowers and Viebrock have commented that the sudden arrival of dust in November 1963 coincided with a change in the stratospheric circulation from circumpolar to meridional. The volcanic dust observations are consistent with the presence of an equatorial reservoir with gradual depletion by enhanced poleward transfer during the winter.

The different times of occurrence of the maxima of the various tracers are probably associated with the different heights at which the tracers occur and at which the transport takes place. A complete understanding, however, requires considerably more detailed information than we have at present.

We thank Dr. Herbert J. Viebrock for permission to use his unpublished results, and Mr. B. G. Collins for his assistance in analysing the Aspendale radiation data.

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EXPERIMENTAL OBSERVATIONS ON THE STRUCTURE OF COLLISIONLESS SHOCK WAVES IN A MAGNETIZED PLASMA

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THIS article reports measurements of the structure of shock waves propagating through a plasma in a direction perpendicular to an initial magnetic field. The shock wave is produced by a fast linear 'Z-pinch' between electrodes with 100 cm separation in a fused silica tube of 50 cm diameter. The applied electric field of 320 V/cm produces an axial current which rises to 300 k.amp in 0·7 μsec, and flows in a thin annular layer ($\sim$ 1 cm). The resulting pinch forces drive this skin current radially inwards at a velocity exceeding the Alfvén velocity for the undisturbed plasma. This rapid radial compression generates an imploding shock wave which propagates ahead of the driving current layer.

The radial distribution of electron density in the initial plasma has been measured by using a double Langmuir probe and by infra-red interferometry. For the particular case of a hydrogen plasma with central electron density $n_e = 7 \times 10^{14}$ cm^{-3} and initial axial magnetic field $B_{z_0} = 1{\cdot}2$ kG, the total density (ions and neutrals) has also been measured. This was obtained from the measured velocity of propagation of a low-frequency, small amplitude, magnetosonic wave. The deduced degree of ionization is more than 85 per cent for the central 20 cm diameter.

The plasma collapse has been examined in detail for the foregoing conditions by using magnetic probes. The shock front, a sharp jump in axial magnetic field strength, is clearly separated from the thin skin current (Figs. 1 and 2). For most of the implosion the Alfvén Mach number (shock velocity (V_S)/initial Alfvén velocity), $M_A = 2{\cdot}5$. The measured magnetic fields have been compared with numerical computations in which the plasma is treated in the magneto-hydrodynamic (MHD) fluid approximation[1]. With this model the shock structure broadens rapidly as viscous effects increase. If viscosity is omitted the structure steepens as the electron temperature rises and resistivity decreases. These structure effects, which disagree with the experimental results, are avoided by introducing into the computation an artificially fixed shock width[2]. Good agreement is then obtained between the experimental and computed dynamics if it is assumed that the neutral gas in the outer 10 cm takes part in the collapse (Figs. 1 and 2).

The structure of the shock front has been examined in detail using magnetic probes of diameter down to 0·9 mm and a coaxial double electric probe with an effective tip of 0·25 mm and variable electrode separation. The electric probe records the radial voltage (V_R) through the shock

front. For the foregoing conditions ($M_A = 2{\cdot}5$), the radial voltage rises in less than 6 nsec as the shock moves on to the tip of the electric probe. The magnetic probe records a coincident but slightly slower rise of magnetic field. Typical oscillograms are shown in Fig. 3. The measured radial voltage (V_R) and change of magnetic field ($B_z - B_{z0}$) in the shock front are plotted in Fig. 4*a* against time for 9 cm radius. As the measured shock velocity is sensibly constant at $2{\cdot}5 \times 10^7$ cm/sec, Fig. 4 also represents the spatial variation.

The measured shock width (1·4 mm) is appreciably shorter than the appropriate ion–ion and ion–neutral mean free paths (5 cm and 2·5 cm, respectively). Consequently, collisional viscosity can be neglected as reported previously[3]. Resistive dissipation through the azimuthal current in the shock front, which is the only other collisional process (electron-ion collisions) capable of sustaining such a shock[4], cannot be eliminated by such a simple criterion. The measured 6-nsec rise time of the voltage is less than two electron-ion collision times. The latter is calculated for the region behind the shock by assuming that the shock heating goes entirely to the electrons, raising their temperature to 50 eV. It is unlikely that this energy can be lost from the shock front in such a short time. As several collisions are required within a collision-dominated shock front it is probable that a collisionless resistivity, such as is provided by turbulence from electron drift instabilities[5,6], is important. The measurements indicate an electron drift velocity of about 10 per cent of the predicted electron thermal velocity. This would correspond to the critical drift velocity for instability given by Stringer[7] if $T_i \sim 0{\cdot}1\ T_e$. The shock width is about $7(c/\omega_{pe})$ for the initial conditions. This is appreciably less than $25(c/\omega_{pe})$ found by Adlam[6]. As further evidence for collisionless interaction, the measured rise time of 13 nsec in deuterium at about the same mass density and M_A corresponds to only half an electron-ion collision time while the shock width is about $10(c/\omega_{pe})$.

The change in directed energy of an ion passing through the shock front (considered at rest) is $\Delta W = \frac{1}{2} M[V_s^2 - (V_s - V_p)^2]$, where M is the ion mass and V_p is the plasma velocity behind the shock (laboratory co-ordinates). The latter has been derived from the measured magnetic field profiles using the fact that (B_z/n_e) is constant on both sides of the shock (though not within it). The measured azimuthal electric field E_θ behind the shock also provides a measure of $V_p (= E_\theta/B_z)$. The measured voltage across the shock is such that the ratio $R = eV_R/\Delta W = 1$. This means that the ion motion through the shock is entirely accounted for by electric field effects. By implication, the change of ion pressure across the shock is negligible. The measured V_R can be accounted for, in accordance with the generalized Ohm's law[8], by the known force ($J_\theta \times B_z$) acting on the electron current (J_θ) and the predicted electron pressure gradient.

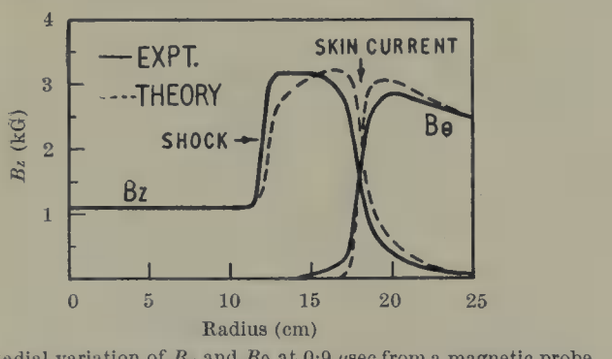

Fig. 1. Radial variation of B_Z and B_θ at 0·9 μsec from a magnetic probe of 7 mm diameter and from computation with a 7 mm shock width

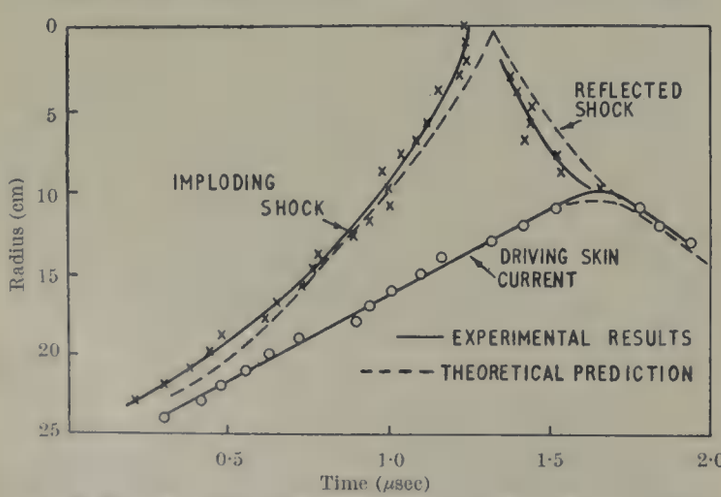

Fig. 2. Space-time 'streak' diagrams of plasma collapse from magnetic probes and computation

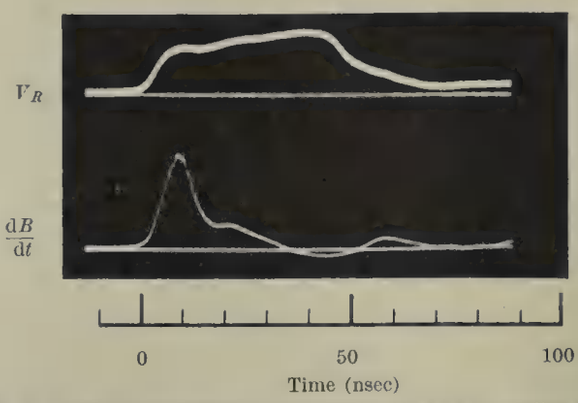

Fig. 3. Typical oscillograms of V_R and dB_z/dt at 9 cm radius for $M_A = 2{\cdot}5$. Electric probe tip separation, 1 cm

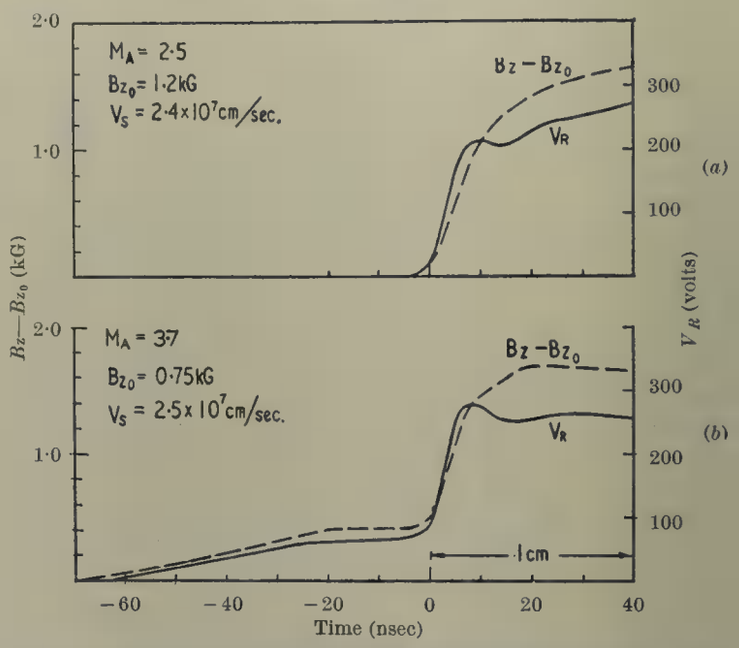

Fig. 4. Structure of magnetic field and electric potential through the shock front for $M_A = 2{\cdot}5$ (top); $M_A = 3{\cdot}7$ (bottom)

With resistivity (either collisional or not) as the only dissipative mechanism, continuum theory predicts the occurrence of a discontinuity (iso-magnetic jump) in the fluid velocity within the shock structure for M_A greater than a critical value $M_A^* \sim 3$ (ref. 4). Physically beyond this limit slip of field and plasma (resistive dissipation) alone cannot provide sufficient dissipation of energy. Theories based on particle orbits predict the occurrence of multiple streaming of the ions above a similar limit[9].

Experimentally M_A is increased by reducing the initial magnetic field. For $M_A = 2{\cdot}5$ and 3·7 the initial plasma density and the shock velocity are effectively the same. However, at $M_A = 3{\cdot}7$ the shock has a clear double structure (Fig. 4*b*) with the sharp transition preceded by a broad one ($\sim 1{\cdot}5$ cm) corresponding to $2c/\omega_{pi}$. This broad feature of the structure appears for $M_A \geqslant 3$ over a range of parameters and is interpreted as evidence for the additional mechanism required theoretically. The

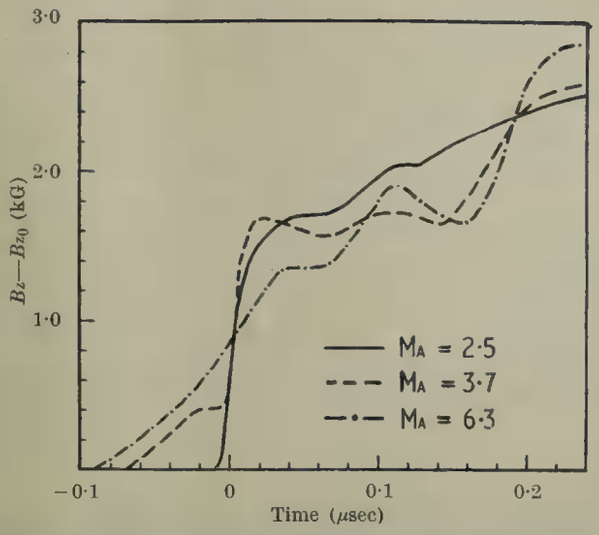

Fig. 5. Structure of magnetic field through the shock front for $M_A = 2{\cdot}5$, 3·7 and 6·3. (For the latter $B_z = 0{\cdot}43$ kG, $n_2 = 4{\cdot}5 \times 10^{14}$ cm^{-3} $V_s = 2{\cdot}8 \times 10^7$ cm/sec)

measured voltage across the shock front again corresponds to $R = 1$, implying that the electrons are heated.

At still higher M_A, the broad structures forward and to the rear of the sharp transition become dominant and at $M_A = 6$ (Fig. 5) the sharp transition is absent. The total structure is then so broad that cylindrical effects are important.

The broad additional feature is shorter than the ion–ion mean free path but is comparable with the ion–neutral mean free path. Even so, this latter mechanism can be neglected because at least five such collisions are required to collect the undisturbed ions (> 80 per cent ionized). Electron thermal conductivity, another relevant collisional mechanism[10], is made negligible by the presence of the axial magnetic field[11]. Consequently, all collisional mechanisms appear to be eliminated for $M_A > M_A^*$.

Of the many theories of collisionless shocks, only a few are valid for $M_A > M_A^*$. Theories involving ion–ion streaming instabilities[12,13] can be eliminated because they predict a sharp transition of width $L \sim \frac{2\pi V_s}{\omega_{pi}}$. The theory of Colgate[14], involving Landau damping of electron oscillation, also predicts a sharp transition with $L \sim \frac{c}{\omega_{pe}}$. Auer *et al.*[15] treat the problem of ion streaming without instabilities but derive an effective entropy increase from ion gyration phase mixing and assume that the electrons are not heated. This approach leads to a time-dependent structure with $L \sim \pi M_A \frac{c}{\omega_{pi}}$ (ω_{pi} for compressed plasma) and $R = 0{\cdot}25$ (for $M_A = 6$). Carmac *et al.*[16] describe a model based on non-linear wave–wave interaction within a front of width $L \sim \frac{2c}{\omega_{pi}}$ for $M_A \sim 4$ but steepening with increasing M_A. The particular analysis, though not the mechanism, is limited to low M_A ($< M_A^*$). Although no explicit prediction is made, it is reasonable to assume that the waves involved ($\omega \gg \omega_{ci}$) heat the electrons and consequently $R = 1$. These values of L and R are comparable with those of the broad feature, but the dependence on M_A appears inconsistent.

Experimental imperfections make it possible that the magnetic field makes a small angle ($\theta > \sqrt{\frac{m_e}{M_p}} \sim 1°$) to the shock front. For the foregoing conditions with $M_A > M_A^*$ this leads to a shock structure[17,18] more than an order of magnitude shorter than that observed and consequently has been neglected.

In summary, at low Mach numbers ($M_A < 3$) the results indicate that a resistive shock is formed which probably involves collisionless turbulence. Above the predicted critical M_A (~ 3) the observed change of structure is interpreted as due to an additional, but unidentified, collisionless mechanism. None of the present theories of shock structure gives convincing agreement over the range of observations, but some can be eliminated.

We thank Dr. R. J. Bickerton for his advice and Mr. C. A. Steed for his assistance.

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PLEISTOCENE GLACIAL-MARINE ZONES IN NORTH ATLANTIC DEEP-SEA SEDIMENTS

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SINCE Bramlette and Bradley[1] first described glacial marine sediment zones in deep-sea cores across the North Atlantic, there has been no further attempt to use these zones as criteria for interpretation of Pleistocene climates. The occurrence of glacial erratics in the North Atlantic has been described by several authors[2–6]. One of the most recent accounts was given by Pratt[6], who described erratic boulders of granite, schist, diorite and quartzite from cores and dredges on the Great Meteor Seamount. These accounts indicate that icebergs floated much farther south in the North Atlantic during the cold periods of the Pleistocene, dropping considerable continental material as far south as 30° N.

An investigation was made of the occurrence of ice-rafted material in the Lamont cores from the North Atlantic to find cores in pelagic sediment which contained large variations in the vertical distribution of ice-rafted debris. In general, all cores taken in pelagic sediment north of 50° N. contained abundant glacial debris. In many areas near Labrador, Greenland and Iceland there was so much glacial debris that obvious variations in the amount of intensity of ice-rafting could not be measured. Farther south, cores described by Ericson *et al.*[7] consist of zones of sediment containing abundant ice-rafted grains.

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Cores taken south of 35° N. contain little or no ice-rafted grains. Hence it was decided to examine the variation in ice-rafted material in the cores between 40° and 50° N., particularly since Ericson *et al.* had already shown that they contained zones consisting of different Foraminifera assemblages deposited during cold and warm periods of the Pleistocene. The zones delineated by Ericson *et al.* can also be defined using the relative abundance of ice-rafted detritus present in the cores.

The amount of ice-rafted detritus present in the sand fraction of 13 cores taken from the North Atlantic (Fig. 1) previously described by Ericson *et al.*[7] was estimated at intervals down the cores (Fig. 2) by examination of the sand fraction under a binocular microscope. The cores lie between latitudes 40° and 50° N. and consist almost entirely of pelagic sediment containing varying percentages of pelagic organisms. Cores *A*157–5 and *A*180–14 were taken near the edge of the Sohm Abyssal Plain and contain one or two very thin quartz silt beds, presumably deposited by turbidity currents; but the rest of the sediment in these two cores is mainly pelagic. The remainder of the cores is located in positions in the deep-sea floor favourable for pelagic deposition.

In general, the sand fraction consists mainly of tests of pelagic Foraminifera mixed with ice-rafted sand and minor amounts of volcanic ash. The ice-rafted detritus is easily identifiable, as it characteristically consists of very poorly sorted, and mainly angular, sand and pebbles of a variety of mineral and rock types. The size, poor sorting, general angularity and wide lithological range of this detritus that occurs in pelagic sediment, far from land, indicate that it has dropped from melting ice. Many of the large pebbles are striated or faceted and large quartz grains are commonly frosted and pitted. Quartz is most abundant and commonly makes up 50–70 per cent of the sand fraction. Pebbles of granitic, gneissic, regional metamorphic and sedimentary rocks of all kinds make up the bulk of the fraction coarser than 1 mm.

Black pumice and brown and black glass occur associated with ice-rafted sand in cores from the eastern North Atlantic. Since the amount of black volcanic detritus present bears a constant relation to the amount of poorly sorted ice-rafted sand, it must also be ice-rafted. The black pumice and glass are most abundant in cores taken close to Iceland (Fig. 1), as has been previously noted by Ericson *et al.*[8], and the amount of black pumice decreases southwards, indicating dilution of this volcanic detritus from Iceland with detritus from eastern Greenland and the British Isles. In the western North Atlantic, quartz is less common and makes up only 30–50 per cent of the ice-rafted sand. Large quantities of hornblende and garnet are particularly characteristic of the sand in cores *A*157–5 and *A*157–6.

The amount of ice-rafted sand present increases from a few grains in the southernmost cores to 20–30 per cent of the total volume of sediment in the glacial marine zones in cores *SP*10–1 and *SP*9–3. The variation in the percentage of ice-rafted detritus in the sand fraction at intervals down the cores (Fig. 2) can be interpreted as a measure of the effects of climate on deposition. Zones containing a relatively high percentage of ice-rafted detritus correlate very closely with zones containing

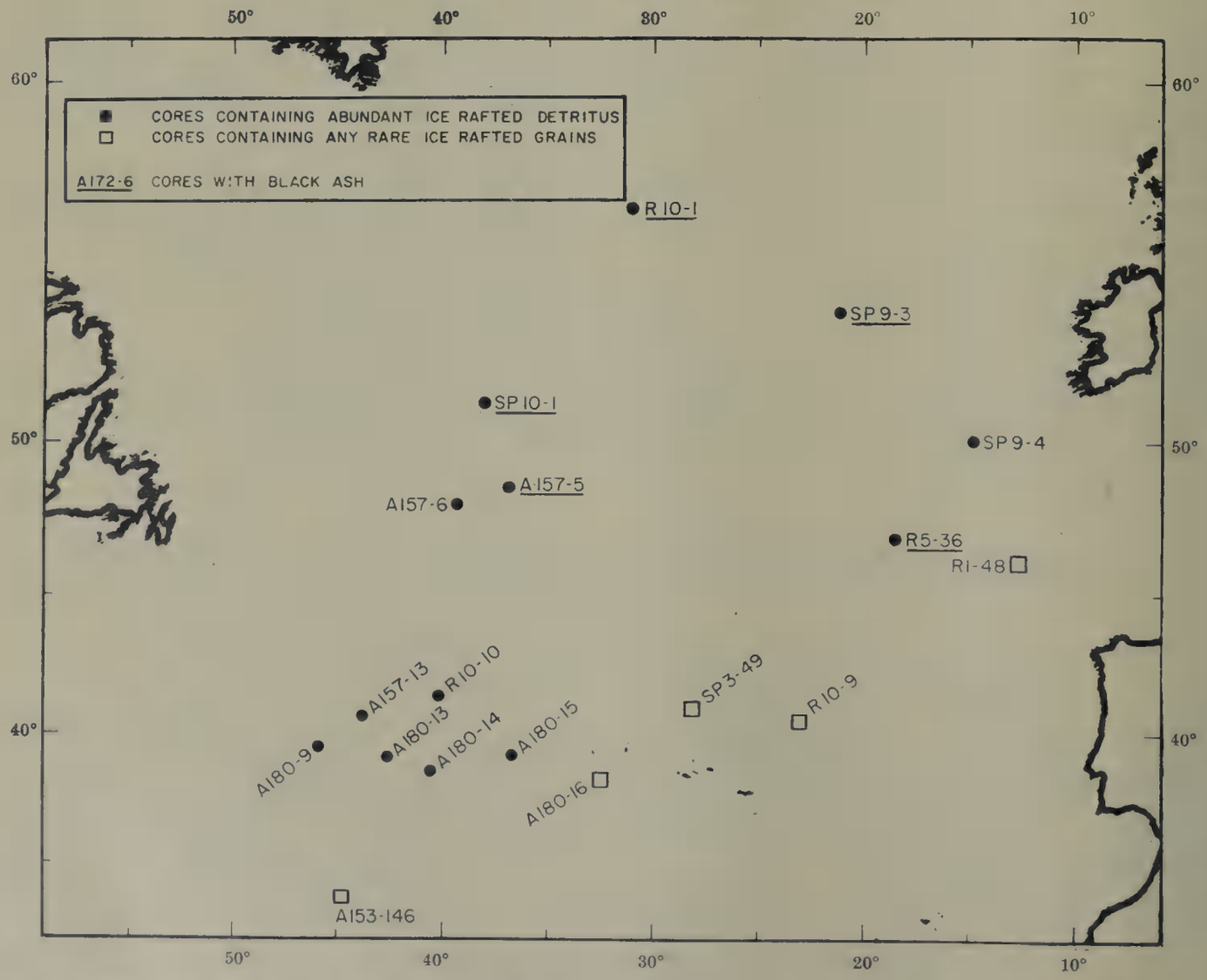

Fig. 1. North Atlantic Ocean showing the location of cores described in this investigation with abundant ice-rafted sand and pebbles and the location of cores containing only rare ice-rafted grains. Cores in the north and north-western North Atlantic contain black pumice and brown and black glass probably derived by ice-rafting from near Iceland

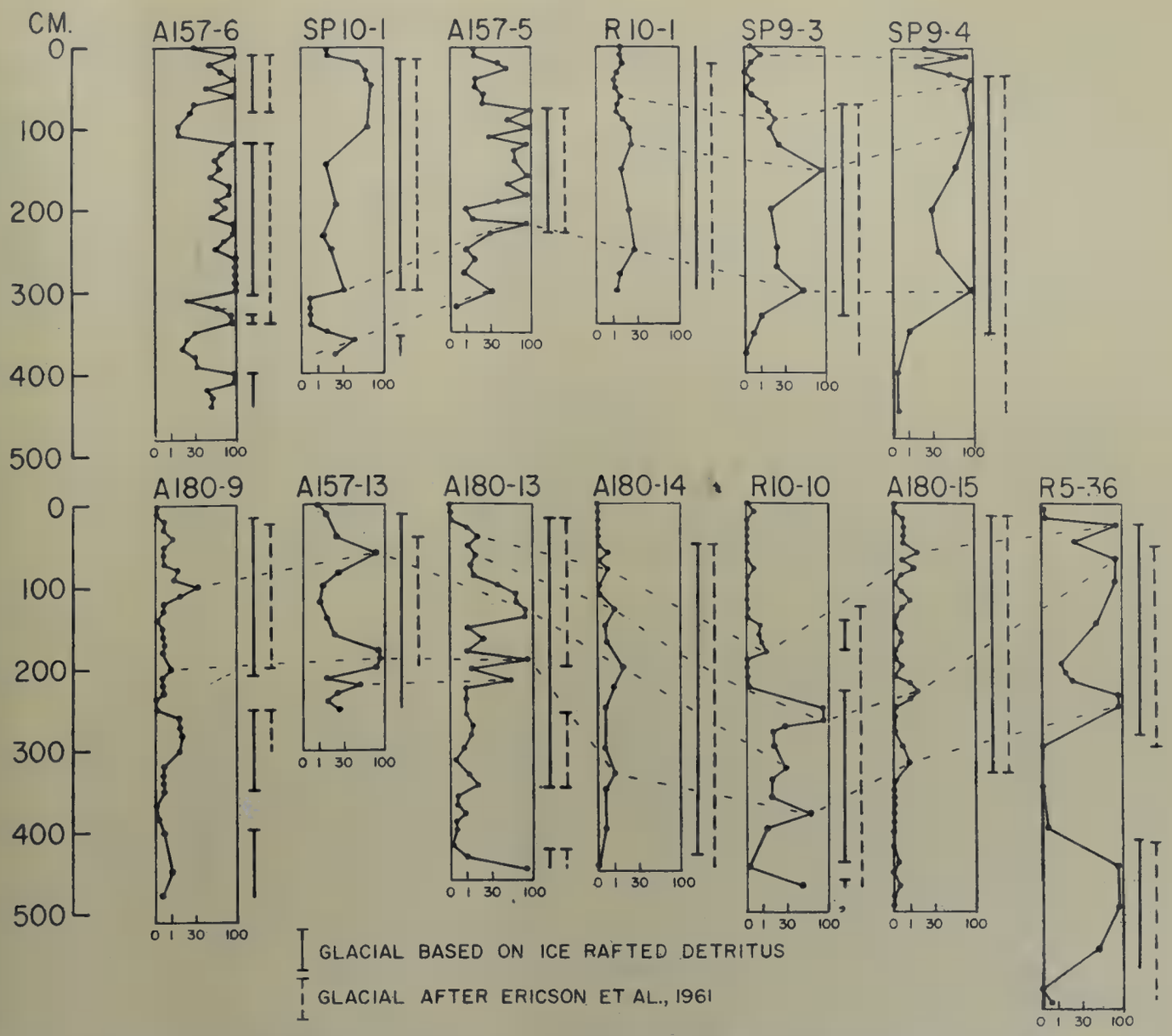

Fig. 2. The amount of ice-rafted detritus in the sand fraction is expressed as a percentage of the total sand fraction at intervals down 13 cores from the North Atlantic. Zones defined as glacials by Ericson *et al.* (ref. 7) in these cores can also be defined on the relative abundance of ice-rafted detritus. Tentative correlations are made between areas of cores containing high amounts of ice-rafted detritus. These areas may represent deposition during the major cold periods of the late Pleistocene

colder-water foraminiferal assemblages described by Ericson *et al.*[7].

There is considerable fluctuation in the amount of ice-rafted detritus deposited during these cold periods, suggesting that it might be possible to determine the major cold and warm zones within each glacial by correlating areas representing maximum and minimum amounts of ice-rafted detritus in different cores across the North Atlantic.

If it is assumed that the zones which Ericson *et al.*[7] considered to have been deposited during the last glacial (the period from about 11,000 to 60,000 years ago), and shown in Fig. 2, are correct, then it is possible to sub-divide further this cold period on the basis of the relative abundance of ice-rafted detritus. For example, core *R*10–10 contains a distinct zone containing abundant ice-rafted detritus between 150 and 180 cm from the top of the core. This zone occurs between areas dated as 11,800 ± 480 and 15,820 ± 200 years old[7] and probably contains ice-rafted detritus deposited during the last major glaciation of the last glacial period. Beneath this zone there are three more zones in which the sand fraction is made up almost entirely of ice-rafted detritus, suggesting that there were probably three more major cold periods during the last glacial period. Similar zones containing high amounts of ice-rafted detritus occur in many of the other cores and possible correlations between them are shown in Fig. 2.

Table 1. Location of Cores used for Ice-rafting Distribution Analyses (Fig. 2)

Core	Latitude	Longitude	Water depth (metres)
*A*157–5	48° 35′ N	36° 51′ W	4,500
*A*157–6	48° 03′ N	39° 20′ W	4,500
*A*157–13	40° 34′ N	43° 51′ W	4,680
*A*180–9	39° 27′ N	45° 57′ W	4,060
*A*180–13	39° 08′ N	42° 39′ W	4,880
*A*180–14	38° 41′ N	40° 40′ W	5,020
*A*180–15	39° 16′ N	36° 42′ W	4,610
*R*5–36	46° 55′ N	18° 35′ W	4,500
*R*10–1	56° 47′ N	31° 00′ W	2,375
*R*10–10	41° 24′ N	40° 06′ W	4,755
*SP*9–3	53° 53′ N	21° 06′ W	2,745
*SP*9–4	50° 02′ N	14° 46′ W	4,205
*SP*10–1	51° 23′ N	38° 04′ W	3,695

These results indicate that the abundance and distribution of ice-rafted detritus in deep-sea cores in the North Atlantic could provide a powerful tool for delineating even minor fluctuations in the Pleistocene climate. Cores taken in pelagic sediment in the vicinity of cores *R*5–36, *R*10–10 and *A*180–13 in particular should contain easily determinable ice-rafted zones that might eventually lead to a better understanding of the fluctuations in climate and rates of sedimentation during the Pleistocene in the North Atlantic. Furthermore, it may be possible to delineate the Pliocene–Pleistocene boundary, using the first appearance of ice-rafted detritus in deep-sea pelagic cores as a criterion. The disappearance of ice-rafted

detritus with depth occurs in sediment cores raised from the deep ocean floor surrounding Antarctica[9,10] and occurs just beneath the Pliocene–Pleistocene boundary based on radiolarian assemblages[11].

So far, none of the cores taken in the North Atlantic ice-rafting area passes by continuous section through the Pleistocene and none has been identified as containing the Pliocene–Pleistocene boundary; but further attempts to take such cores are now in progress.

We thank D. B. Ericson, who kindly lent us samples of sand from Lamont cores. Important support for this work was provided by a Ford Foundation fellowship and a Fulbright travel grant for Dr. J. R. Conolly. This work was also supported by the Office of Naval Research and by the U.S. National Science Foundation.

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AN INFRA-RED INVESTIGATION OF THE EFFECT OF PRESSURE ON SILICA POWDERS, AS REVEALED BY DEUTERIUM OXIDE EXCHANGE

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IN recent years, infra-red spectroscopic techniques have been widely used to examine the surface properties of a variety of solids. The direct examination of finely divided solids is hampered by scattering of the incident radiation. This problem is often overcome by pressing self-supporting disks which have much higher transmission and, additionally, have a higher number of functional groups present in the beam. Infra-red investigations of the exchange properties of the surface hydroxyl groups present on silica, with deuterium oxide (D_2O) vapour, show that the high pressures used in disk formation can significantly alter the behaviour of the sample.

The pretreatment and surface properties of the rehydrated 'Aerosil' used in the work recorded here have been described elsewhere[1]. Disks were prepared from about 50-mg samples which were placed in a die and evacuated (on a conventional vacuum-line fitted with liquid nitrogen traps) to a pressure of 2×10^{-4} mm for 30 min. Two disks, *RA*2/5 and *RA*2/10, were formed by pressing at 5 tons/cm² and 10 tons/cm² respectively for 30 min. Two further disks, *DA*2/5 and *DA*2/10, were pressed after six-fold exchange in the die with saturated D_2O vapour (Norsk Hydro, purity 99·7 per cent); each contact lasted 120 sec and the sample was evacuated to 2×10^{-4} mm mercury between each exchange, before final evacuation for 30 min at the same pressure.

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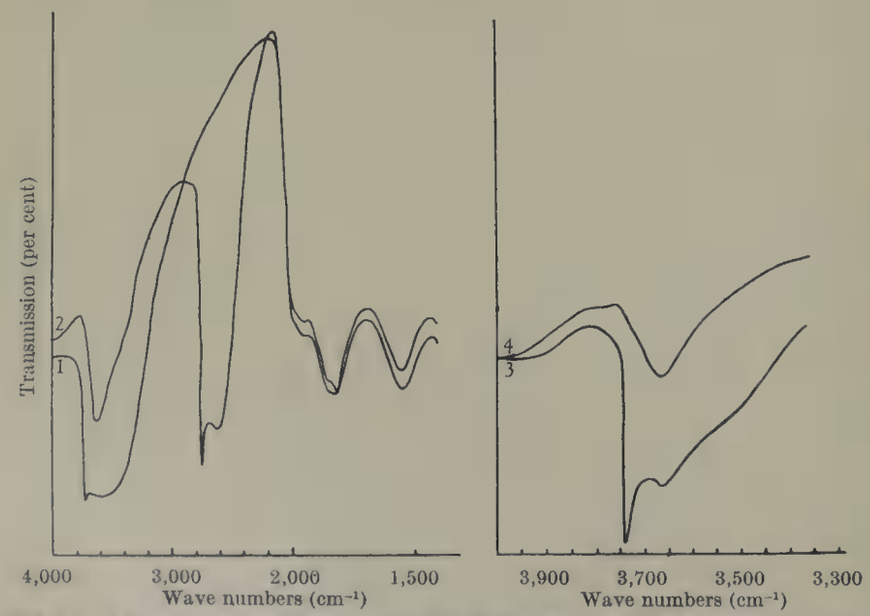

Fig. 1. (1) Infra-red spectrum of original disk *RA*/5; (2) spectrum after D_2O exchange; (3) infra-red spectrum of original powder *RA*—ordinate expanded 50 times; (4) spectrum after D_2O exchange

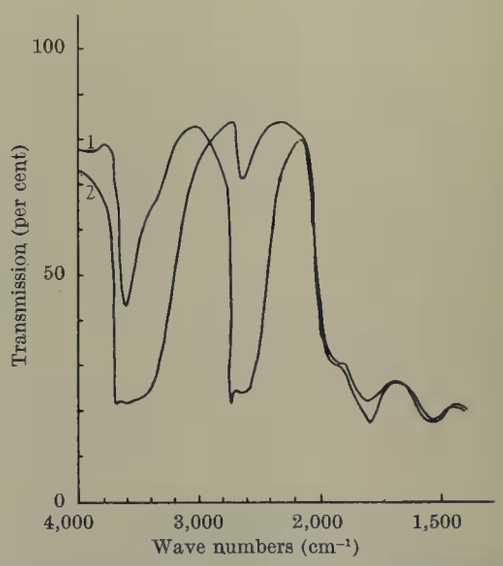

Fig. 2. (1) Infra-red spectrum of disk made from deuterated powder—*DA*/5; (2) spectrum after H_2O exchange

The disks were mounted in a stainless-steel cell, similar to that described by Harrison and Lawrence[2], and spectra were recorded between 4,000 cm⁻¹ and 1,500 cm⁻¹, using a Perkin–Elmer 125 double-beam grating spectrometer. The cell was connected to a vacuum line fitted with facilities for treating the sample, *in situ*, with water and heavy water vapour. Disks *DA*2/5 and *DA*2/10 were further treated with D_2O vapour to replace the surface OD groups lost during the transfer of the sample from the die to the cells.

The spectra of *RA*2/5 before and after D_2O exchange are given in Fig. 1; these show that an appreciable proportion of the 3,650 cm⁻¹ hydroxyl band is unchanged; this proportion is even greater in the case of an *RA*2/10 disk. The absence of a peak at 3,737 cm⁻¹ indicates that all the single hydroxyl groups are exchanged. Similar spectra for the unpressed *RA*2 powder are also shown; these indicate that a proportion of the unexchangeable hydroxyl groups are present in the powder.

That additional unexchangeable surface groups result from compression is illustrated by the spectra given in Fig. 2 for

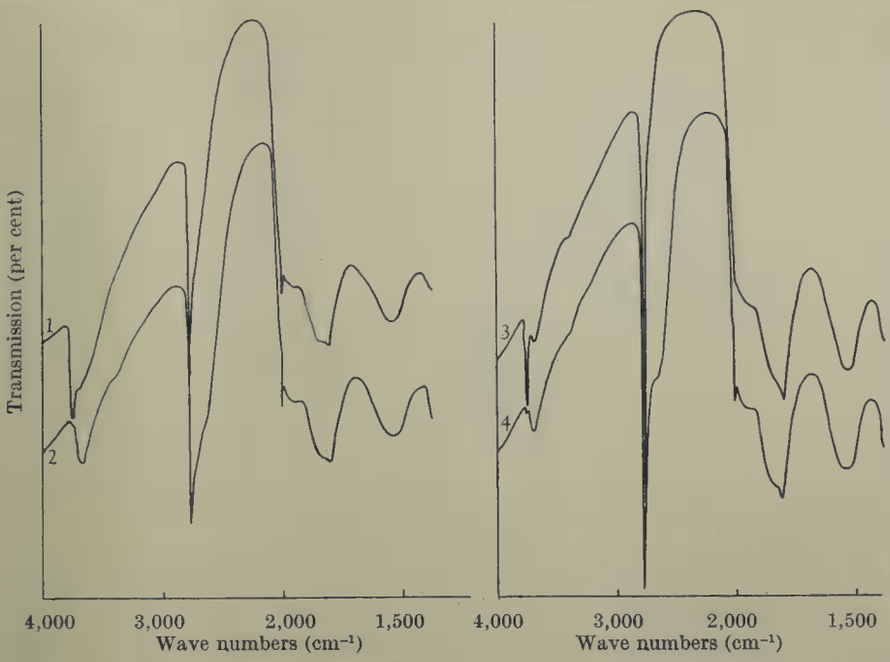

Fig. 3. (1) Disk *RA*/5 heated to 300° C; (2) after D_2O exchange; (3) disk *RA*/5 heated to 450° C; (4) after D_2O exchange

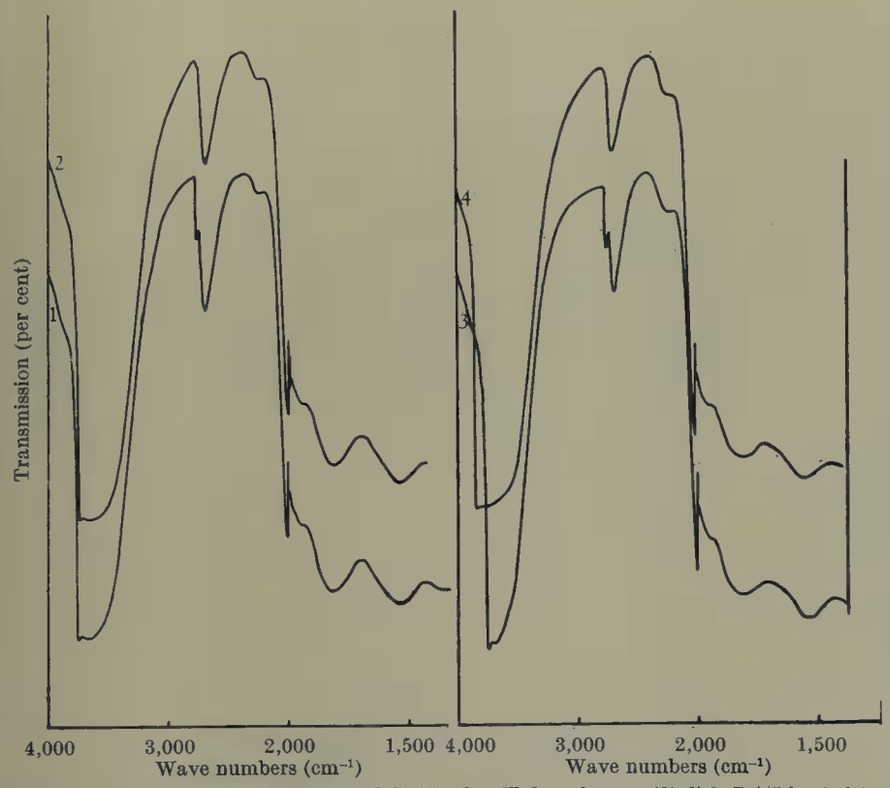

Fig. 4. (1) Disk *DA*/5 heated to 150° C; (2) after H_2O exchange; (3) disk *DA*/5 heated to 300° C; (4) after H_2O exchange

*DA*2/5. As with the powder sample, there are unexchangeable hydroxyl groups, but in addition there are also OD groups resulting from exchange of the powder, characterized by a band at 2,710 cm^{-1}, which cannot be exchanged with water vapour. The intensity of this band is greater for *DA*2/10 than for *DA*2/5. Control experiments show that all exchanges carried out on the disks are reversible. No change in the 1,640 cm^{-1} band as a result of the exchange was noted.

Figs. 3 and 4 show respectively the effects of heating the exchanged *RA*2/5 and *DA*2/5. Surface dehydration is accompanied by a decrease in intensity of the low-frequency component of the vibrational absorption bands for both the exchanged and unexchangeable groups. However, there is an increase in the absorption intensity of the 3,737 cm^{-1} band, assigned to single hydroxyls, for *RA*2/5, and in that of the 2,755 cm^{-1} band, assigned to single deuteroxyls, for *DA*2/5. These new 'single' absorption bands can now be readily changed, together with some of the low-frequency tail. When samples are heated to higher temperatures, exchange is accompanied by a large degree of chemisorption even with contact times of < 60 sec at room temperature. The spectrum of the D_2O-exchanged *RA*2/5, after heating to 450° C, was stable after further evacuation for 60 h, showing that the growth of the 3,737 cm^{-1} band did not result from leakage of the cell.

The spectral results presented show that formation of a silica disk results in a product having certain important differences from the original powder. It is clear that it may be erroneous to make direct comparison between the infra-red results obtained from such pressed disks, and gravimetric and adsorption data determined on the corresponding powders. The evidence suggests that either regions inaccessible to H_2O or D_2O vapour are formed, or that bringing the particles closer together causes enhanced hydrogen bonding which prevents exchange occurring. However, as it is thought that it is the most closely adjacent OH groups which are first lost on dehydration, a fact confirmed by the more rapid decrease in the low-frequency tail of the ν_{OH} band, then such strongly bonded groups would be expected to be more rapidly lost than the exchangeable hydroxyl groups. This is shown not to be the case. Additionally, the increase in the single ν_{OH} (or ν_{OD}), observed on heating, would not be expected by this mechanism. Disks made at lower pressures exhibit the same effects, but to a lesser extent. It is realized that pressures described elsewhere for the preparation of 'Aerosil' disks are sometimes lower; however, 'Aerosil' probably represents an optimum case and other oxides require higher pressures.

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HEXAGONAL-MESH STRUCTURES AND STABLE POLYPEPTIDE CHAIN CONFIGURATIONS

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HECHTER[1] has recently proposed a hexagonal-sub-unit model for the protein component of cell membranes, based on polypeptide chain structure principles set forth by Warner[2,3]. However, available experimental and theoretical evidence shows such structures to be exceedingly unlikely, and the basis for the membrane model therefore should be reconsidered.

Warner has proposed that simple peptide antibiotics such as gramicidin *S* and actinomycin are built up from a basic hexapeptide unit in which the carbonyl oxygens

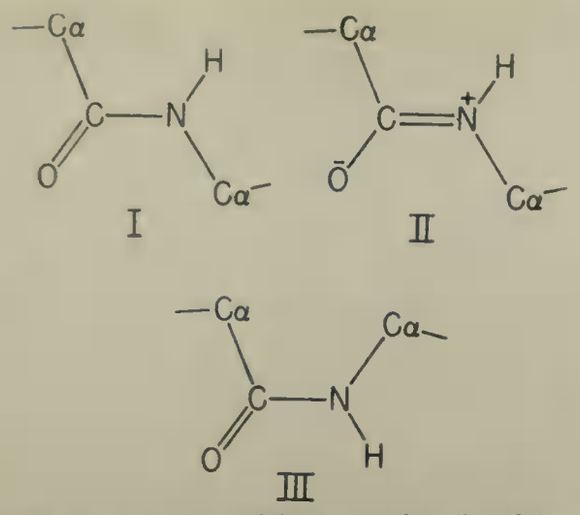


Fig. 1. Two resonance forms of the *trans* configuration of the planar peptide bond (I and II) and the *cis* configuration (III)

lie 4·8 Å apart at the corners of a hexagon. The hydrophobic side-chains project from one face of the flat molecule in this model, and the hydrophilic carbonyl and NH groups extend from the other face. Decapeptide antibiotics are then related to the basic hexamer as naphthalene is to benzene, and larger units are built up on a flat hexagonal mesh. The two essential features of the model are the separated hydrophilic and hydrophobic sides, and the regular 4·8 Å hexagonal spacing of carbonyl oxygens on the hydrophilic face, related to the second-neighbour oxygen spacings in the structure of ice. This flat hexagonal mesh model has been extended to the 158-residue protein sub-unit of TMV as well[4], although the stacking model shown does not agree well with electron micrographs of the TMV molecule.

Hechter's membrane model uses flat spiral hexagonal sub-units of a similar size packed in an overlapping double layer with hydrophobic faces paired between layers, and with channels or pores through the membrane. Several properties of the membrane are then explained with considerable ingenuity from this model. There may well be elements of truth in the cell membrane model, but the peptide hexamer principles on which it is based are energetically untenable.

The fundamental flaw in the peptide model is that it cannot be built with the desired properties without deforming the amide or peptide linkages severely out of their stable planar configurations (Fig. 1). In contrast, there is considerable evidence for the true planarity of peptide bonds in peptides and proteins.

Theoretical Evidence

Early structural work on small compounds by Corey and Pauling[5,6] and others showed the amide bonds to be uniformly planar. Pauling explained this in terms of the joint contribution of two resonance structures, I and II in Fig. 1. The central C—N bond would be shortened from its normal 1·475 Å as it assumed a partial double-bond character and the C=O distance would increase beyond 1·205 Å as it lost double-bond character. Assuming a 60 : 40 contribution of I and II, the expected C—N distance was calculated to be 1·32 Å and the expected C=O distance to be 1·24 Å.

The resonance stabilization energy of the amide bond has been calculated by Pauling and Sherman[7a,b] from

Table 1. Results of the X-ray Analyses of Simple Compounds containing Peptide Bonds
The order is roughly chronological, with references only to the most recent or most complete analyses

Compound	C–N distance in Å	C–N per cent double-bond character (*a*)	C=O distance in Å	Max. deviation from amide planarity (*b*)	*S.D.* in bond-lengths in Å (*c*)	Ref.
Acetamide	1·38		1·28	—	—	13
α-glycylglycine	1·32	35	1·24	Planar	—	14
β-glycylglycine	1·29	40	1·23	< 6°	—	15
N-acetylglycine	1·32	39	1·24	< 1·3°	—	16
L-glutamine	1·28	58	1·27	—	0·024	17
Urea	1·34	28	1·26	—	0·012	18
Glycyl-L-tyrosine hydrochloride	1·35	~25	1·16	0·20 Å	~0·03	19
Glycyl-L-asparagine	1·32	35	1·23	< 0·01 Å	0·014	20, 26
N-*N*′-diglycyl-L-cystine dihydrate	1·35	25	1·21	6°	~0·03	21
Formamide	1·30	45	1·26	—	0·018	22
Oxamide	1·315	39	1·243	—	0·005	23
N-*N*′-diacetylhexamethylene diamine	1·35	25	1·22	0·01 Å	~0·006	24
Glycyl-L-tryptophan dihydrate	1·33	32	1·23	0·02 Å	0·014	25
Succinamide	1·333	33	1·238	—	0·002	26
L-leucyl-L-prolyl-glycine	1·34	42	1·27	0·04 Å	0·015	27
	1·31	38	1·24	0·03 Å		
Glutathione (γ-L-glutamyl-L-cysteinyl-glycine)	1·31	~42	1·23	0·12 Å	0·028	28
	1·32	~38	1·24	0·05 Å		
Diketopiperazine	1·325	~35	1·239	0·010 Å	0·002	29
N-methyl acetamide	1·29	~50	1·24	0·015 Å	0·013	30
Glycyl-phenylalanyl-glycine	1·39	(*d*)	1·21	0·034 Å	0·02	31
	1·34	~30	1·23	0·011 Å		
Cu(II) monoglycylglycine trihydrate	1·277	~55	1·264	Planar	0·016	47
	1·275	~55	1·277	Planar	0·014	
Tosyl-L-proline-L-hydroxyproline monohydrate	1·32	~35	1·24	6·5°	—	32
*Cyclo*hexaglycyl hemihydrate	1·34	~29	1·23	~0·05 Å	—	48
Glycylglycylglycino Cu(II) chloride sesquihydrate	1·304	~42	1·228	0·057 Å	0·012	49
	1·307	~41	1·190	0·024 Å		
Sodium glycylglycylglucino cuprate(II) monohydrate	1·29	~50	1·23	0·05 Å	0·012	50
	1·36	(*e*)	1·24	0·16 Å		
Disodium glycylglycylglycylglycino cuprate decahydrate	1·290	~50	1·276	0·015 Å	0·006	33
	1·316	~39	1·267	0·004 Å		
	1·301	~45	1·272	0·013 Å		
Potassium benzylpenicillin	1·33	~34	1·25	Planar	(*f*)	34

(*a*) Per cent double-bond character is taken from Hahn (ref. 8), based on both C–N and C=O bond-lengths. Later approximate values are interpolations based on C–N distances only. The limits of error in these figures are wide. The difference in bond-length between complete single-bond character and complete double-bond character is only about 0·24 Å, and an uncertainty (three standard deviations) of 0·03 Å in bond-length would correspond to an uncertainty in per cent double-bond character of ±12 per cent.

(*b*) Maximum deviation in Å of any atom from the best least-squares plane fitted to the amide group, or alternatively, angle of twist about the C–N bond in degrees. For small angles of twist, β, it is approximately true that: $\sin\beta = 0{\cdot}8\,d$, where d is the deviation in Å of one of the corner atoms of the amide plane from co-planarity. The greatest observed non-planarity is 0·20 Å or 9°, corresponding to a strain energy of only 0·5 kcal/mole.

(*c*) Standard deviations in most cases have been obtained from least squares refinement. There is a 95 per cent chance that a variation of as much as three times the standard deviation is not statistically significant. This should be kept in mind when assessing limits of error in the per cent double-bond figure.

(*d*) The value of only 15 per cent double-bond character obtained from this 1·39 Å bond-length is misleading. The authors themselves state that, because of experimental errors in the data, the difference of 0·07 Å between this value and the expected one of 1·32 Å is not significant (ref. 31).

(*e*) The second C–N bond is badly distorted in the crystal because of the chelation of all three nitrogens to the same copper atom. Note that the C=O bonds, farther removed from the copper atom, are as expected for about 40 per cent double-bond character. In spite of the considerable lengthening of the second C–N bond this amide is only twisted 7·4° out of planarity.

(*f*) No standard deviations are given, but single bonds are observed to have a mean variation of 0·03 Å from their expected values.

(*g*) Cysteinyl glycine sodium iodide has been reported (ref. 11) as having a non-planar amide. The original structure analysis is definitely in error and the data are being re-analysed by Dr. J. Donohue (ref. 12).

(*h*) For further discussions of amide planarity, see refs. 8, 9 and 10.

thermodynamic heats of formation of formamide, acetamide and oxamide. Values of 21·4, 20·5 and 23·7 kilocalories per mole of amide bonds, respectively, were obtained. In view of the similarity of C=O and C—N bond-lengths in these compounds and in peptides, the resonance energy per amide in a polypeptide chain is unlikely to differ from 21 kcal by more than a few kcal. A reasonable measure of the strain energy involved in twisting an amide linkage out of planarity about its C—N bond by an angle β is then: $E = 21 \sin^2\beta$ kcal/mole. Hence even a twist of 30° will require a strain energy of more than 5 kcal.

Experimental Evidence

The experimental evidence for the planarity of the peptide bonds falls into three categories: di- and tripeptides and related compounds, fibrous proteins, and globular proteins. The results of careful X-ray analysis of compounds in the first category are given in Table 1. In all cases the amide bond has been found to be planar, and in all the peptides it is in the *trans* configuration (I rather than III in Fig. 1). In all cases the expected shortening of the C—N and lengthening of the C=O bond are found, and the contribution of resonance form II usually lies in the 30–40 per cent range.

These observations are given added significance by the X-ray examination of potassium benzylpenicillin, a larger molecule containing one amide bond[34]. The bond is *trans* and planar, and showed the expected changes in bond-lengths (Table 1).

In X-ray analyses of fibrous proteins, accurate bond parameters are not sought, and analysis consists essentially in fitting sterically acceptable models to the observed diffraction pattern[35]. Nevertheless, the field only began to produce results after the work of Corey *et al.* had produced standard parameters for polypeptide chain geometry[5,6]. In all work since then, whether with α-helices, β-sheets, collagen triple helices or more complex structures, it has never been necessary to invoke non-planar amides to satisfy the diffraction pattern, and in only one case (polyproline I (ref. 36)) have *cis* amides been found.

Complete structure analyses are only beginning to appear for globular proteins, and the only ones at near-atomic resolution (2 Å or better) so far are myoglobin[37,38] and lysozyme[39]. Two-angstrom resolution does not permit measurement of accurate atomic parameters, but geometry of the peptide links is clear. In all cases, for both myoglobin[51] and a preliminary examination of lysozyme[52], only *trans* amides were found, planar within the limits of accuracy of the structure (of the order of ± 5° for myoglobin). The fact that such restrictions are observed in both a high-helix and a moderately low-helix protein, and in both hydrophobic and hydrophilic regions of the molecules, suggests that violations of planarity in other structures will be difficult to justify.

In summary, there is no evidence from any structure analysis, from simple dipeptides to proteins, that the amide bond ever deviates more than a few degrees from planarity, and in all but two or three small cyclic compounds such as diketopiperazine and cyanuric acid and one fibrous polypeptide only the *trans* configuration has been found.

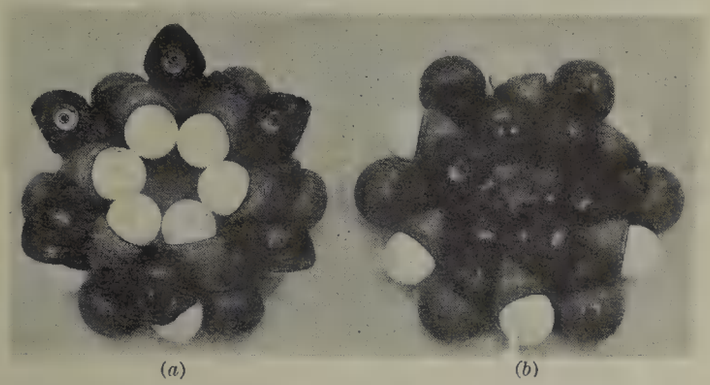
Hexagonal Peptide Models

In contrast to the foregoing evidence, the hexagonal-unit peptide models proposed by Warner[2–4] require the amide bonds to be deformed out of planarity, in some instances by nearly 90°. Of the eighteen amide bonds in a nonadecapeptide fragment of ACTH[1], only one appears to be *trans* and planar. Four are planar but *cis*, four are skew but nearer *cis* than *trans*, seven are skew but nearer *trans* than *cis*, and two appear to be at right angles. A rough calculation of strain energy based on Pauling's formula, and assigning twist angles no more precisely than 0°, 45°, 90°, 135°, and 180° to the categories mentioned here as determined from the photograph, gives a value of about 160 kcal/mole.

As part of an X-ray analysis of actinomycin[41] we have tried building Warner's actinomycin molecule shown in Fig. 6*b* of ref. 2. The total strain energy in this molecule, based on amide deformations measured from the model and accurate to ± 10°, is 120 kcal/mole. An unstrained configuration with eight planar *trans* and two planar *cis* linkages is possible in a model of the same size and shape as Warner's (Fig. 11, ref. 41). However, the structural advantages of Warner's model are largely lost. Only six of the ten carbonyl oxygens extend out from the 'hydrophilic' face, two extend out from the edges and two more now project from the 'hydrophobic' side. Nothing at all resembling a regular hexagonal array of carbonyl oxygens can be seen. Extensive adjustments of the space-filling models have shown no way to segregate hydrophobic and hydrophilic groups or to re-create the oxygen arrangement without deforming the amides from planarity.

If the planarity of the amide bond is accepted as a fundamental principle, then the larger the structure the more difficult it becomes to retain the specific features of Warner's structures. The basic hexapeptide with *cis* amides (Fig. 2*b*) has α-carbons so close that the structure is only usable for hexaglycine. The *trans* hexamer (Fig. 2*a*) does place the carbonyl oxygens 4·8 Å apart in a hexagon and extend the side-chains out the opposite side. However, the NH groups fall on the 'hydrophobic' rather than the 'hydrophilic' side.

The decapeptide built up from two fused hexagons is less tractable. The planar *trans* model is shown in Fig. 3 with a bond sketch in the corner. The two re-entrant angles at positions 1 and 6 pose a problem. The amide linkage considered as a unit does not alter the direction of the polypeptide chain (Fig. 4*a*), and any bending of



(*a*) (*b*)

Fig. 2. Hexapeptide models with planar amides, in (*a*) the *trans* and (*b*) the *cis* configuration. Side-chains are represented only by single carbon atoms attached to the main chain or α-carbon. Note the steric hindrance between these side-chain carbons in the *cis* form

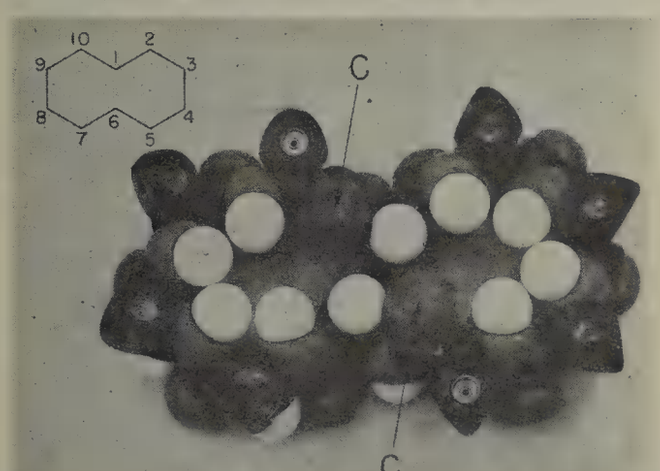


Fig. 3. Decapeptide with planar *trans* amide bonds, showing the 'hydrophobic' side. Side-chains are represented only by single (β) carbons. Note the presence of the two carbonyl groups (*c*) on the 'wrong' side of the molecule

the chain must be produced at the α-carbons (Fig. 4*b*, *c*). At re-entrant angles the C_α must be rotated 180° from the position of Fig. 4*b* to 4*c*, bringing the side-chain over to the hydrophilic side, where it collides with the carbonyl oxygen.

At any re-entrant angle, therefore, one must either move the side-chain of one residue to the hydrophilic side or replace the L-amino-acid by a D-form. If the L-form is retained, then the adjacent amide group must be rotated so as to move the carbonyl oxygen to the hydrophobic side to get it out of the way unless the residue involved is glycine. Fig. 5 shows the decapeptide with the carbonyl rotated in this manner to the 'wrong' side, and Fig. 6 shows the steric hindrance between carbonyl and side-chain if this is not done. A brief examination of sequences of decapeptide antibiotics[42] shows that except for gramicidin *S* (and actinomycin if it is one decapeptide ring), one does not generally find D-amino-acids or glycines paired across the ring.

Larger structures become increasingly difficult. Three rings of Warner's flat spiral TMV structure (Fig. 1 of ref. 4) were built, and the preceding comments about re-entrant angles were checked. A further extension of model building was not felt to be worth while. No less than 64 of the 158 residues in the TMV structure are involved in re-entrant angles in this model, meaning that almost as many carbonyls and side-chains will project from each side unless many of these re-entrant sites are glycine or D-amino-acids. The known amino-acid sequence of TMV (ref. 43) lends no support to this latter idea.

Energy Requirements for Non-planar Amide Structures

It has recently been suggested[44,45] that, even though the non-planar amide structures (hereafter referred to as 'strained' structures) were not the lowest energy states for small peptides and antibiotics in solution, these structures might be stabilized by hydrogen bonding and hydrophobic interactions when in contact with enzyme

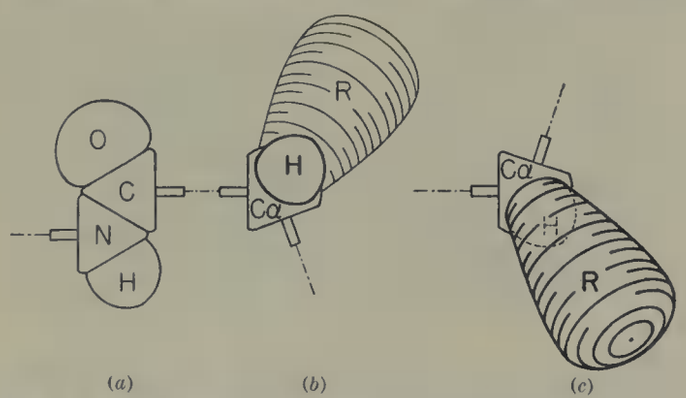


Fig. 4. Space-filling models of: (*a*) planar *trans* amide group, (*b*) and (*c*) α-carbon with hydrogen (*H*) and side-chain (*R*). Note that any change in chain direction must occur at an α-carbon site, as the planar *trans* amide group taken as a unit leaves the chain direction unaltered

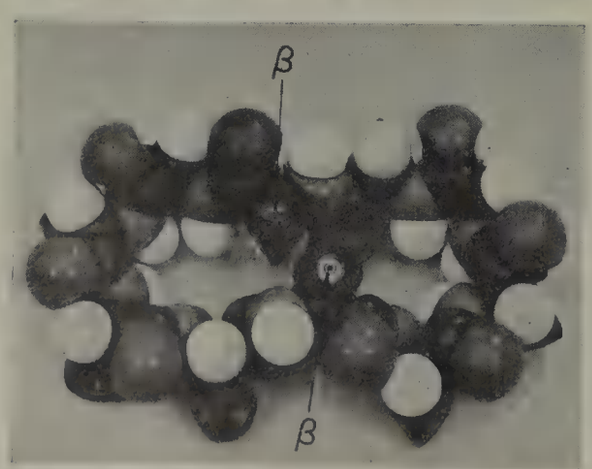


Fig. 5. Decapeptide of Fig. 3, showing the 'hydrophilic' side. Note the presence of two side-chains (β, represented by β-carbon atoms only) on the 'wrong' side, and the fact that there is barely room for them

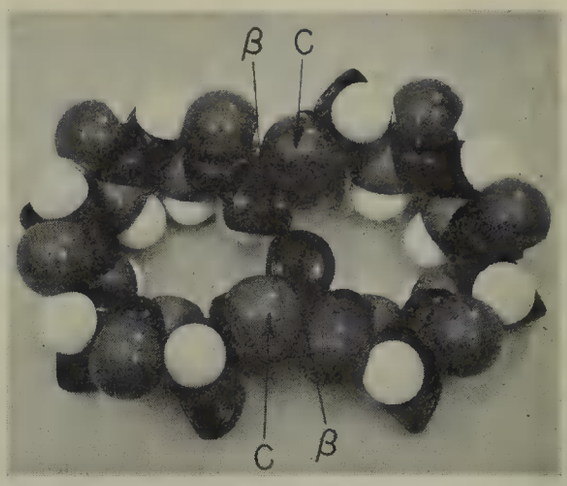


Fig. 6. Decapeptide of Figs. 3 and 5, 'hydrophilic' side, with the two displaced carbonyls (*c*) rotated back to the proper side. Note the severe steric hindrance between these two carbonyls and the neighbouring side-chains (β)

surfaces, cell membranes or even like molecules in the form of dimers. By this argument, two peptide configurations, each inherently unstable when the units are isolated, would become a stable system when the units were locked together through the collective effect of a number of weak forces. This is a perfectly valid concept if it is made quantitative, but dangerous if it is not. Undoubtedly the exact conformation of a polypeptide chain can be strongly influenced by binding to a supporting surface or to a smaller substrate molecule. But it is not difficult to show that neither hydrogen bonding nor hydrophobic interactions are sufficiently strong to compensate for the strain energies enountered in these non-planar amides.

In such a quantitative comparison, it is not enough to compare the amide strain energy with the total energy obtained by hydrogen bonds and hydrophobic interactions, for an unstrained polypeptide configuration can always be found with much the same binding properties if not the same precise configuration (as with the two actinomycin models). One must therefore compare the strained molecule in a given set of circumstances with an unstrained molecule in an equivalent environment.

(1) *Average strain energy per amide bond.* With a fragment of ACTH containing 18 amide bonds, a crude strain energy estimate of 160 kcal was obtained. The actinomycin figure of 120 kcal for ten amides is more accurate. Thus, although there is a maximum possible strain energy of 21 kcal per bond for bonds wrenched to right angles, an estimate of 10 kcal per bond as an average in hexagonal-type structures is certainly safe. If such strained structures are to be stable, some factors must be found which will contribute an average energy of at least 10 kcal per amino-acid residue.

(2) *Stabilization by favourable hydrogen bonding with solvent.* If the same number of hydrogen bonds can be formed with the strained and unstrained structures, then there is no stabilization. But now suppose that a fraction y of the possible bonds in the strained form are impossible in the unstrained form. A simple inversion of a carbonyl or NH group from one face of the molecule to the other is not sufficient, and to be inaccessible the group must be buried. Examination of models suggests that certainly not more than 30 per cent of the possible groups will be so buried.

The enthalpy of formation of a hydrogen bond with water is about −5 kcal. The entropy change is less precise, but it will certainly be negative, making the free energy change greater than −5 kcal (less in absolute magnitude). The exact value does not matter, for the conclusions hold even if one takes the least favourable case and neglects the entropy entirely. In this case, the stabilizing contribution of the extra hydrogen bonds in the strained form will be $\Delta F = -5y$ kcal per mole of

amide bonds, or −1·5 kcal at the most. This is quite insufficient to counteract the free energy change of +10 kcal per bond in going to the strained form.

(3) *Stabilization by pairing of hydrophobic faces.* Pairing of hydrophobic faces is energetically favourable not because of van der Waals interactions between hydrophobic side-chains, which amount to only a few tenths of a kilocalorie, but because of the removal of such groups from the aqueous environment. Kauzmann[46] has shown that the most important term involved is the entropy one, and has calculated that the removal of one hydrophobic side-chain from water to a non-polar environment is accompanied by a free-energy decrease of no more than about 4 kcal.

Let us assume that in the strained model all hydrogen-bonding and hydrophobic groups are properly segregated to their respective sides of the flat molecule, while in the unstrained version a fraction x of each type is on the wrong side. The free energy of stabilization of the strained molecules by pairing of hydrophobic faces will be $\Delta F_{h,s} = -4$ kcal per amino-acid residue. In the unstrained molecule, only a fraction $(1-x)$ of the hydrophobic groups will be buried, for a stabilization energy of $-4(1-x)$ kcal. But a fraction x of the previously formed hydrogen bonds will be broken because they occurred to groups on the 'hydrophobic' face, producing a destabilization of $+5x$ kcal in the most unfavourable circumstances. The net free energy of stabilization is then $\Delta F_{h,u} = -4+9x$ kcal per mole. The lowest reasonable value of x is 0·5, that for a random distribution of types of groups between faces.

The net free energy change in going from unstrained to strained molecules, both with the maximum possible use of hydrophobic interactions, is then:

$$\Delta F = \Delta F_{\text{strain}} + \Delta F_{h,s} - \Delta F_{h,u} = (+10-4+4-9x)$$

kcal per bond.

If the worst possible case is assumed, comparing a completely ordered strained molecule with an unstrained molecule having random occurrence of side-chains on either side, then $x = 0{\cdot}5$ and $\Delta F = +5{\cdot}5$ kcal.

The conclusion is therefore that even if the most favourable hydrogen bonding and hydrophobic interactions are used, the flat, hexagonal-mesh, non-planar-amide structures are unstable relative to a planar-amide form by 5–10 kcal per amide bond.

Summary

The hexagonal-mesh models proposed by Warner, if made with planar amides, do not create distinct hydrophobic and hydrophilic sides and do not place the carbonyl oxygens at the corners of a hexagonal mesh 4·8 Å on a side as proposed. Moreover, the strain involved in twisting the amides out of planarity is so great that even the best hydrogen bonding and hydrophobic bonding cannot make these strained structures stable. If models of this general type are proposed, they should be based on planar (and probably *trans*) amide groups, and tested with space-filling models rather than hexagonal-mesh diagrams, for that which is true for single or double rings is not necessarily extendable to a semi-infinite mesh.

It is probably reasonable to say that any peptide model with non-planar amides can be regarded from the outset as extremely improbable. But if it proves useful for certain purposes to hypothesize models of this type, then their instability must be kept clearly in mind, and at some point a quantitative numerical justification for such a distorted structure must be made. At present there is no direct evidence for the hexagonal-mesh structures considered here and overwhelming evidence against them.

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A NEW INHERITED COAGULATION DISORDER CAUSED BY AN ABNORMAL FIBRINOGEN ('FIBRINOGEN BALTIMORE')

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An inherited abnormality of fibrinogen resulting in an asymptomatic clotting defect was described by Ménaché[1]. Another congenital qualitative defect of fibrinogen associated with a symptomatic coagulation disorder has been discovered in a 29-year-old woman of Anglo-Saxon origin[2]. The patient was admitted to the Johns Hopkins Hospital because of femoral vein thrombosis following minor trauma. She had a history of a

Table 1. Coagulation Studies

	Patient %	Normal control %
Fibrinogen assays:		
(*a*) Thrombin clotting time (ref. 3).	35 mg	175 mg
(*b*) Tyrosine determination (ref. 4)	104 mg	180 mg
(*c*) Immunodiffusion (ref. 5)	180 mg	180 mg
Prothrombin time (one-stage)	45	60–100
Prothrombin time after addition of normal fibrinogen	80	
Specific assays for factors II, V, VIII, IX, XIII	Normal	
Euglobulin lysis time	Normal	
Platelet count	224,000–284,000	
Platelet function (aggregation and clot retraction)	Normal	

mild haemorrhagic diathesis characterized by frequent bruising, epistaxis and menorrhagia, and a history of a previous severe thrombotic episode of several months' duration.

On preliminary investigation a friable whole blood clot was noted. Assays of fibrinogen (Table 1) indicated the presence of a poorly clottable fibrinogen in the patient's plasma: subnormal values were obtained by a method that depends on the rate of clotting in the presence of standard amounts of thrombin[3] whereas the amount of immunologically detectable fibrinogen was within normal limits. Further studies of coagulation demonstrated that fibrinogen alone was responsible for the clotting disorder. An abnormally prolonged prothrombin time was corrected by the addition of normal fibrinogen to the patient's plasma. Fibrinolytic activity as measured by euglobulin lysis was not increased. Recalcified plasma clots were insoluble in urea, indicating that factor XIII (fibrin-stabilizing factor) activity was present.

The rate of clotting of the patient's plasma by thrombin[1] (Thrombin Topical, Parke, Davis and Company, Detroit, Michigan) was retarded, especially at low concentrations of thrombin (Fig. 1). Addition of calcium chloride (0·025 M) partially corrected the prolonged thrombin clotting time of the patient's plasma but had little effect on the thrombin clotting time of normal plasma.

The rate of clotting of normal plasma or fibrinogen by thrombin was not inhibited by the patient's plasma or by her fibrinogen. In contrast to acquired defibrination syndromes due to intravascular coagulation, the clotting defect was not corrected by therapy with heparin and infused normal fibrinogen had a normal survival time in the patient's circulation.

The venom of *Bothrops jararaca* ('Reptilase', Pentafarm, Basle, Switzerland), which is known to clot fibrinogen by splitting off predominantly peptide *A* (ref. 6), did not clot the patient's prothrombin-free plasma. On addition of 'Reptilase' and calcium ions a clot formed, but at a comparatively slow rate (Table 2).

The thromboelastogram (prepared by Dr. S. Attar, University of Maryland, Baltimore, Maryland) showed an abnormally small maximal amplitude (Fig. 2). The rate of clot lysis was not increased. In the absence of platelet abnormalities an abnormal structure of the final clot was therefore suggested.

The patient's fibrinogen could not be distinguished from normal fibrinogen on paper electrophoresis and so far consistent differences of migration have not been observed on starch-gel electrophoresis. On immunodiffusion using rabbit antibody prepared against normal human fibrinogen, the precipitation lines that formed in the presence of normal and patient plasma fused without observable spurring (Fig. 3). However, on immunoelectrophoresis minor but reproducible migration differences were found. The abnormal fibrinogen, therefore, appeared to be immunochemically similar to, but not identical with, normal fibrinogen.

Table 2. Clotting Time of Prothrombin-free Citrate Plasma by 'Reptilase'

	Plasma (ml.)	'Reptilase' (ml.)	Calcium 0·025 M (ml.)	Buffer (ml.)	Clotting time (sec.)
Normal:	0·1	0·05	—	0·05	53
	0·1	0·05	0·05	—	27
	0·1	—	0·1	—	No clot*
	0·1	0·05	—	0·05	No clot*
Patient:	0·1	0·05	0·05	—	84
	0·1	—	0·1	—	No clot*

* Observed for 24 h.

These experiments indicated that the defect represented a dysfibrinogenaemia rather than hypofibrinogenaemia, and suggested that the disorder might be inherited. Investigation revealed the presence of a similar abnormal fibrinogen in all three daughters of the proposita, whereas the two sons had normally clottable fibrinogen. Two of the daughters had a history of a significant bleeding tendency. The disorder appears to be transmitted by a dominant gene (Fig. 4). No further abnormal family members were found in three generations of the patient's mother's family. The father's family was not available for investigation.

Qualitative abnormalities of fibrinogen may be overlooked, particularly if the defect does not produce symptoms. The defect described by Ménaché[1] was discovered in a patient with no haemorrhagic symptoms. In our proposita and in two of her children an abnormal bleeding tendency is clearly present. It remains to be shown

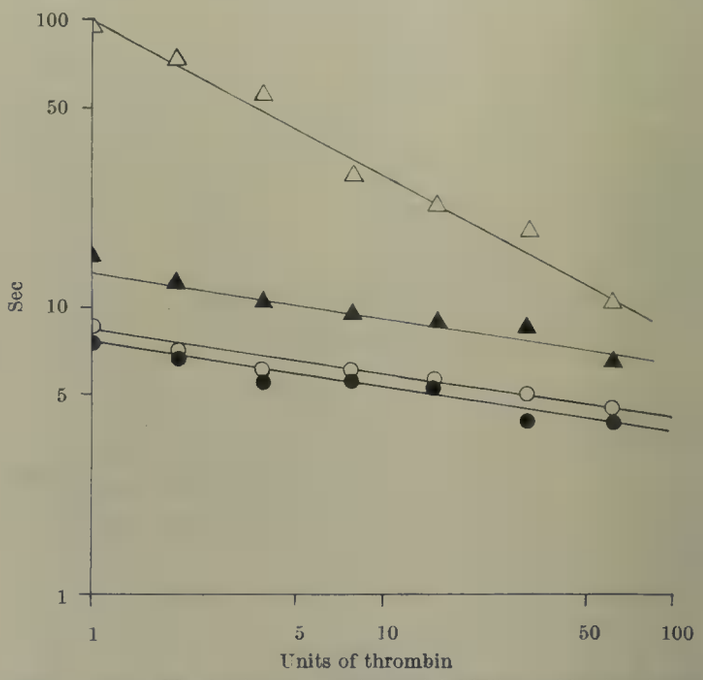

Fig. 1. Thrombin clotting times using serial dilutions of bovine thrombin were performed on normal and patient citrated plasma. Thrombin was diluted with barbital buffer (*p*H 7·35) alone or with 50 vol. per cent 0·050 M $CaCl_2$ and barbital buffer. ○, Normal + thrombin + buffer; ●, normal + thrombin + calcium; △, patient + thrombin + buffer; ▲, patient + thrombin + calcium

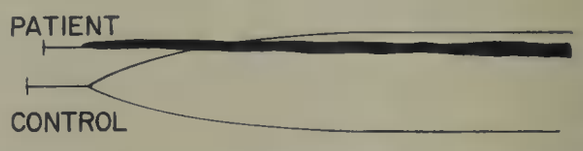

Fig. 2. Thromboelastogram on re-calcified platelet-rich citrated plasma. Maximal amplitude: normal, 34·0 mm; patient, 4·5 mm

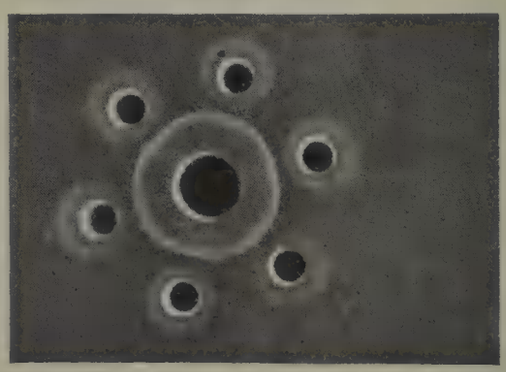

Fig. 3. Immunodiffusion studies. Centre well: antibody against normal fibrinogen. Peripheral wells: citrated plasma, undiluted. Normal plasma and patient plasma are in alternate wells. Complete fusion, shown by a smooth ring, is present indicating antigenic identity in this system

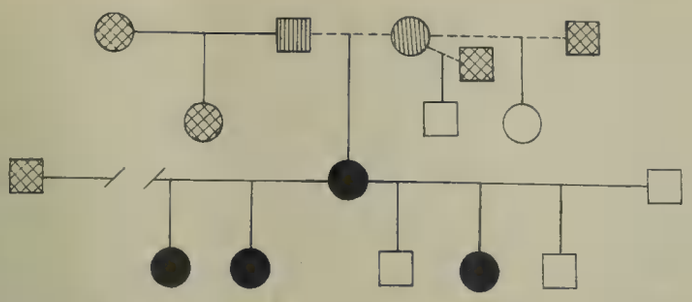

Fig. 4. Family distribution of abnormal fibrinogen. White, tested, normal; black, abnormal; cross-hatched, not tested; hatched, deceased

whether the thrombotic episodes are related to the defective fibrinogen.

The exact sites of the structural defects in the abnormal fibrinogens described by Ménaché and by us are not known. In contrast to the fibrinogen described here, the abnormal fibrinogen described by Ménaché interfered with the conversion of normal fibrinogen by thrombin, suggesting that the two abnormal proteins may be dissimilar. It has been shown that the amino-acid sequence of the peptides which are split off from fibrinogen by thrombin affects the interaction between thrombin and fibrinogen[7]. The absence of clot formation of our patient's fibrinogen in the presence of 'Reptilase' suggests a possible abnormality of peptide A. The effect of calcium ions in partially correcting the clotting defect cannot be explained on the basis of our present knowledge.

The abnormal fibrinogen described here is, so far, characterized as follows: (1) it clots at an abnormally slow rate in the presence of thrombin; (2) in the absence of calcium ions it does not clot on addition of 'Reptilase'; (3) calcium partially corrects the defect in the presence of both thrombin and 'Reptilase'; (4) the structure of the final clot is abnormal, but the transamidation mechanism induced by factor XIII appears not to be involved; (5) the abnormal fibrinogen does not interfere with clotting of normal fibrinogen; (6) it can be distinguished from normal fibrinogen by differences in migration on immunoelectrophoresis; (7) its presence is controlled by an abnormal dominant gene and leads to a mild haemorrhagic disorder.

As a consequence of the rapidly increasing knowledge concerning the structure of fibrinogen and the fibrinopeptides it can be assumed that further structural abnormalities may be found in the future. Some of the cases which have been thought to have a dominantly inherited hypofibrinogenaemia[8] may in fact be found to have a dysfibrinogenaemia. A suggested nomenclature to distinguish molecular changes of a protein is that in use for the characterization of abnormal haemoglobins. Accordingly, we would like to designate the fibrinogen described here as 'fibrinogen Baltimore'.

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MECHANISM OF INTESTINAL ABSORPTION: TRANSPORT OF METHIONINE AND SODIUM BUTYRATE BY INTRACELLULAR PLASMA

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A THEORY of the mechanism of intestinal absorption involving active transport by a spectrum of mobile intracellular proteins was put forward in a previous article[1]. This resulted from the analysis of the effects of cetrimide and other protein-binding substances, such as chloroquine and thevetin, on the intestinal absorption of glucose in the mouse during intraluminal perfusion of the small intestine. The main points of the theory are: (*a*) phloridzin acts on a first receptor, blocking a portal of entry for sugars and short-chain fatty acids in the cell membrane; (*b*) phloridzin is attached to a protein moiety of the first receptor, since phloridzin itself can be displaced, and thus inactivated, by high concentrations of cetrimide[2]; (*c*) cetrimide acts on a second, mobile, receptor, which is the one concerned in active transport. Its inhibition results in leakage of blood glucose to the intestinal lumen; (*d*) stimulation and inhibition of absorption can be produced by small and large concentrations, respectively, of substances competing with the nutrient for this second receptor; (*e*) the second receptor is located on intracellular mobile proteins which are characterized by a spectrum of affinities for nutrients and drugs; (*f*) the association–dissociation constants for proteins, drugs and nutrients are controlled by a *p*H or electrochemical gradient, which is maintained by the metabolic activities of the cell and requiring the expenditure of energy.

The localization of the second receptor within the cell membrane was considered, but was rejected as unlikely for two reasons. Effective potentiation of phloridzin by cetrimide, which removes the former from silent receptors, took half an hour to become established. The phloridzin receptor could not, therefore, be on the surface of the cell membrane, for potentiation would have been immediate. Furthermore, the second receptor must lie more deeply still from the surface of the cell, and it seemed inconceivable to accommodate a whole spectrum of mobile proteins and their gradient-establishing enzymes, in addition to the distinct protein of the first receptor, within the 75-Å wide cell membrane, of which some 40 Å consists of a lipoid outer and inner layer.

The general applicability of the theory was next investigated. It was decided to seek parallel evidence for other species and nutrients. The effect of the cationic cetrimide and the anionic sodium lauryl sulphate (B.P.) on the absorption of the amino-acid methionine, the short-chain fatty acid compound sodium butyrate, and glucose, was therefore studied in albino rats. Appreciable inhibition of the absorption of the three nutrients had been demonstrated previously in this species[1], as well as the potentiation of the phloridzin effect on glucose absorption by cetrimide. The important problem of stimulation of absorption by low concentrations of different protein-binding inhibitor drugs had not been investigated in the rat. Stimulation was important, because it indicated mobility of the second receptor[3,4], while its poorly specific interaction with protein-binding drugs indicated its intracellular localization on the mobile proteins.

The drugs were dissolved in 0·9 per cent sodium chloride containing 0·2 per cent (w/v) D-glucose, DL-methionine and sodium butyrate. 50 ml. of solution was perfused through the lumen of the proximal 60 cm of the small intestine for 0·5 h. The percentage absorption of each nutrient was calculated from estimations of their final concentrations. The methods used in the estimations

Table 1. Effect of Cetrimide on the Percentage Intestinal Absorption (Mean ± S.E.) of Glucose, Butyrate and Methionine in the Rat
No. of experiments in parentheses

	Controls	Concentration of cetrimide (w/v)					
		10^{-3}	10^{-4}	$2{\cdot}5 \times 10^{-5}$	10^{-5}	$5{\cdot}0 \times 10^{-6}$	10^{-6}
Glucose	41·2 ± 0·7 (45)	17·4 ± 2·0 (7)	38·1 ± 3·5 (4)	45·1 ± 0·8 (21)	44·8 ± 1·3 (12)	41·9 ± 1·2 (20)	40·7 ± 1·8 (4)
P		≪ 0·001		< 0·001	< 0·05		
Butyrate	43·0 ± 0·9 (40)	33·0 ± 1·1 (7)	44·0 ± 3·1 (4)	45·4 ± 1·2 (8)	45·6 ± 1·5 (10)	46·5 ± 1·3 (20)	43·5 ± 2·2 (4)
P		< 0·001		< 0·3 > 0·2	< 0·2 > 0·1	≏ 0·03	
Methionine	29·4 ± 0·7 (37)	20·3 ± 1·7 (7)	25·3 ± 3·1 (4)	29·5 ± 1·2 (9)	35·2 ± 1·9 (11)	25·4 ± 1·7 (10)	27·4 ± 3·6 (4)
P		< 0·001			≪ 0·001		

Table 2. Effect of Sodium Lauryl Sulphate on Percentage Intestinal Absorption (Mean ± S.E.) of Glucose, Butyrate and Methionine in the Rat
No. of experiments in parentheses

	Controls	Concentration of sodium lauryl sulphate (w/v)									
		$2{\cdot}5 \times 10^{-3}$	10^{-3}	10^{-4}	$7{\cdot}5 \times 10^{-5}$	$5{\cdot}0 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$	10^{-5}	$7{\cdot}5 \times 10^{-6}$	$5{\cdot}0 \times 10^{-6}$	10^{-6}
Glucose	41·2 ± 0·7 (45)	30·9 ± 2·6 (6)	36·0 ± 2·6 (6)	37·2 ± 2·1 (4)	39·9 ± 2·4 (4)	44·0 ± 0·9 (12)	43·6 ± 1·7 (10)	42·8 ± 1·5 (10)	41·9 ± 0·9 (12)	47·1 ± 1·7 (12)	38·4 ± 2·4 (4)
P		≪ 0·001				< 0·1 > 0·05	< 0·2 > 0·1			< 0·001	
Butyrate	43·0 ± 0·9 (40)		33·9 ± 5·7 (6)	51·0 ± 3·7 (4)	50·6 ± 3·6 (4)	49·1 ± 1·8 (11)	49·8 ± 2·9 (10)	47·2 ± 1·7 (10)	47·6 ± 2·0 (12)	47·7 ± 1·3 (12)	43·8 ± 3·3 (4)
P			< 0·01	< 0·02	< 0·02	≏ 0·004	≏ 0·005	< 0·05			
Methionine	29·4 ± 0·7 (37)		17·3 ± 2·9 (6)	24·2 ± 2·3 (4)	26·6 ± 1·2 (4)	36·4 ± 1·7 (12)	34·9 ± 1·8 (10)	30·2 ± 1·5 (10)	35·3 ± 1·6 (12)	33·6 ± 1·9 (12)	32·4 ± 2·2 (4)
P			≪ 0·001			≪ 0·001	< 0·005		< 0·001	< 0·02 > 0·01	

were essentially as before[1], but a slight modification of the methionine estimation requires the reduction of previously published values by a factor of 1·4, before they can be compared with the present values.

The results obtained at six concentrations of cetrimide are shown in Table 1. At a concentration of 10^{-3}, significant inhibition of absorption of all three nutrients occurred, although the inhibition was smaller in each case than that obtained with previous samples of cetrimide[1,2]. At lower concentrations, there was definite stimulation of absorption, which was maximal at $2{\cdot}5 \times 10^{-5}$ for glucose, $5{\cdot}0 \times 10^{-6}$ for butyrate, and 10^{-5} for methionine. At lower concentrations still, absorption returned to its level in control animals.

Sodium lauryl sulphate had only a moderate inhibitory effect on the absorption of glucose compared with that of cetrimide. The results obtained with ten concentrations of sodium lauryl sulphate are given in Table 2. Maximal stimulation of glucose absorption occurred at $5{\cdot}0 \times 10^{-6}$, although there was also some stimulation at $5{\cdot}0 \times 10^{-5}$ and $2{\cdot}5 \times 10^{-5}$. Marked stimulation of butyrate absorption was observed with sodium lauryl sulphate at 10^{-4}–$2{\cdot}5 \times 10^{-5}$, and combination of the four *P* values from the individual *t*-tests in this region gave $P < 0{\cdot}0005$. Stimulation of methionine absorption occurred between $5{\cdot}0 \times 10^{-5}$ and $5{\cdot}0 \times 10^{-6}$, although not at the intermediate concentration of 10^{-5}, which gave a value no different from that of the control.

Although the stimulation of absorption obtained with these two drugs was not as marked as the stimulation of glucose absorption in the mouse, the low *P* values fully establish the stimulating effect at low concentrations for all three nutrients. These results assume greater significance when considered alongside the parallel results in the mouse, since they constitute further confirmatory evidence for the proposed theory of the mechanism of intestinal absorption. The observation that both a cationic and an anionic compound, known to bind protein in an unspecific reaction, stimulate the absorption of the three nutrients at different concentrations, lends strong support to the concept that active transport involves the intracellular proteins which have different affinities for individual nutrients and drugs. It is possible that different nutrients bind to different proteins, but they may also bind to different sites on the same protein.

Inspection of the concentrations at which stimulation occurred with cetrimide shows that for all three nutrients there was a single peak, though the range at which butyrate absorption was stimulated was rather wide. By contrast, stimulation with sodium lauryl sulphate occurred at more than one peak for glucose and methionine, and the range of concentrations for butyrate stimulation was much wider. Both peak multiplicity and range width suggest that more than one protein carrier might be involved, the carriers exhibiting particularly different affinities for sodium lauryl sulphate, but the presence of impurities cannot be excluded.

The first receptor in intestinal sugar transport was previously studied in some detail in the mouse and rat[1,2]. It was concluded that stimulation of sugar absorption in the mouse was due to the action of small doses of a competitive substrate (or inhibitor) on the second, not the first, receptor. This must also be true in the rat, for the results of experiments in the two species are similar. As for sodium butyrate and methionine, little is known about their entry mechanisms. The partial block to butyrate absorption by phloridzin[1,5] may be exerted on one of the entry mechanisms, for butyrate might enter partly through a pore and partly through the lipoid of the cell membrane. For amino-acids, entry through the lipoid part of the cell membrane has been suggested[6], and a two-stage system of absorption has been proposed[7], involving entry and subsequent active transport. The evidence presented here strongly suggests that for all three nutrients stimulation and inhibition of absorption, by low and high concentrations of cetrimide and sodium lauryl sulphate, occur by competition at the second receptor, concerned with active transport. We shall therefore confine our discussion to the various possible modes of stimulation that may be produced by an inhibitor of the second receptor. Since the simple model of carrier transport capable of achieving this was proposed by Wilbrandt and Rosenberg[3,4], other possible mechanisms have been suggested or demonstrated. These different stimulation–inhibition models will therefore be considered to see which of them would be applicable to our findings.

The first is the mobile carrier transport model of Wilbrandt and Rosenberg. According to this, stimulation or inhibition depends on whether the factor $(I' + 1 - S_1'S_2')$ in their formula is negative or positive. Here S_1' and S_2' represent substrate or nutrient concentration at the two ends of the carrier path, and I' is the equilibrium value of the inhibitor substance, all three being divided by the respective equilibrium association–dissociation constant for substance and carrier. This is the model adopted previously in the new theory for the mechanism of intestinal absorption.

The second hypothesis is that of an exchange reaction at a membrane surface in a model of carrier transport

having a 1 : 1 nutrient-carrier stoichiometry (Jacquez[6,8], Wong[6], Guroff[8], Christensen *et al.*[9]). If *C* indicates carrier, the ternary combination *CSI* with subsequent breakdown to *CS* + *I* leads to more rapid formation of *CS* than by direct combination. Employing a more comprehensive scheme for his models, Jacquez obtained equations for which simple analytic solutions could not be found. Consideration of idealized stationary states, however, led him to the conclusion that the simple 1 : 1 nutrient-carrier stoichiometry of Wilbrandt and Rosenberg could not produce stimulation at any concentration of the inhibitor, when the value of S_2, the concentration of the substrate at the inner end of the transport system, remains zero. The same result is obtained by putting zero for S_2 in the Wilbrandt–Rosenberg formula. This state of affairs may conceivably apply for certain cells, such as the Ehrlich ascites cells, if the rate of utilization of *S* by the cell is more rapid than its absorption, but it scarcely applies to the intestinal absorption of nutrients. Furthermore, it seems unlikely that there is ternary complex formation allowing exchange reaction in the case of all three nutrients of the present investigation.

Thirdly, a model of carrier-transport having a 2 : 1 nutrient-carrier stoichiometry was proposed by Jacquez[8]. This allows the formation of *CSS*, *CSI* and *CII* at the same time. The derived equations again permit early stimulation and subsequent inhibition. While this may be the case for some nutrients, it would seem unlikely to represent the standard form for the general carrier-transport reaction.

Fourthly, in a model of polymer enzyme splitting into individual units by low concentrations of the inhibitor substance, Gerhart and Pardee[10] have explained the activation and inhibition of aspartic transcarbamylase by maleate on the hypothesis that the enzyme was composed of four units, forming a tetramer, and that low concentrations of maleate broke the tetramer, enabling the aspartate to become bound to the enzyme units. At higher concentrations, the maleate not only broke the tetramer, but also competed with the aspartate for the enzyme sites, thus acting as an inhibitor. Such a system shows a sigmoidal dose-response curve, while the single enzyme or carrier-transport units, which follow Michaelis–Menten kinetics, show a hyperbolic type of curve. Michaelis–Menten kinetics have been shown to hold for the intestinal absorption of most nutrients of the type studied here[11–13]. That the polymer type of system is general, and would thus apply to all three nutrients, again seems unlikely.

The fifth model involves the facilitation of transfer by increase of oppositely charged groups along pore or path of entry. Rummel and Stupp[14] found that mersalyl stimulated intestinal calcium transport at $5{\cdot}0 \times 10^{-4}$ M and inhibited it at $5{\cdot}0 \times 10^{-3}$ M. They postulated that mersalyl molecules were bound in charged pores, and the increase in anionic groups, thus produced, stimulated calcium transfer. This may evidently be important for ionized particles, but unlikely to constitute a general mechanism of the type observed in this investigation.

In conclusion, analysis of the results reveals the stimulation–inhibition transport pattern to be general in its applicability to species and nutrients. Among the five different possible models, that described by Wilbrandt and Rosenberg is the simplest, and seems the only one that could fit all the results and qualify for a general carrier-transport system with interactions that are not highly specific. The present investigation, therefore, gives a more general significance to the mechanism of intestinal absorption proposed previously, which attributes active transport to a spectrum of intracellular carrier proteins in the cell plasma.

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STOICHIOMETRY OF PROTON TRANSLOCATION THROUGH THE RESPIRATORY CHAIN AND ADENOSINE TRIPHOSPHATASE SYSTEMS OF RAT LIVER MITOCHONDRIA

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ACCORDING to the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation proposed by Mitchell[1–4], the linkage between electron transport and phosphorylation occurs not because of hypothetical energy-rich chemical intermediaries as in the orthodox view, but because oxido-reduction and adenosine triphosphate (ATP) hydrolysis are each separately associated with the net translocation of a certain number of electrons in one direction and the net translocation of the same number of hydrogen atoms in the opposite direction across a relatively ion-, acid- and base-impermeable coupling membrane (see also ref. 5). Thus, the chemiosmotic hypothesis, unlike the orthodox chemical hypothesis, predicts that oxido-reduction through the respiratory chain of the mitochondrial membrane system, and ATP hydrolysis through the membrane-located ATPase, should each be accompanied by the stoichiometric translocation of protons (written $\rightarrow H^+$) across the coupling membrane; and that the P/O quotients observed during respiratory chain phosphorylation should be the product of the $P/\rightarrow H^+$ quotient of the ATPase system and the $\rightarrow H^+/O$ quotient for oxidation of the substrate through the respiratory chain system. It was thought that the direct measurement of $P/\rightarrow H^+$ and $\rightarrow H^+/O$ quotients in rat liver mitochondria would afford a means of testing the chemiosmotic hypothesis, and the present article gives a preliminary report of the results of this quantitative test.

Two main conditions must be satisfied for the accurate measurement of $P/\rightarrow H^+$ and $\rightarrow H^+/O$ quotients: (i) the $\rightarrow H^+$ measured must correspond to a known amount of ATP hydrolysed or of oxygen consumed; (ii) the measurements of $\rightarrow H^+$ must be done in such a way as to minimize the unknown extent of back flow or exchange of H^+ ions and OH^- ions across the membrane system during ATP hydrolysis or substrate oxidation. Estimates of the stoichiometry of H^+ 'secretion' accompanying divalent cation uptake[6,7] or during monovalent cation uptake in presence

of valinomycin or gramicidin[8,9] in steadily respiring or ATP-hydrolysing mitochondria are qualitatively interesting, but cannot be relied on quantitatively because they do not satisfy the foregoing conditions. We have met condition (i) by adding a known small quantity of ATP or oxygen to a lightly buffered anaerobic mitochondrial suspension and recording the consequent change of *p*H (ΔpH) in the suspension medium with a glass electrode during virtually complete hydrolysis of ATP to ADP and P*i*, or virtually complete reduction of the added oxygen by substrate present in the suspension medium. Condition (ii) was fulfilled by using quantities of ATP or oxygen such that the electrochemical potential gradient of H^+ ion developed across the mitochondrial membrane system during the transient ATPase or electron transport reaction was sufficiently small and brief to permit ΔpH recordings to be made before much back flow of H^+ or OH^- ions had occurred.

The buffering capacity of the system that we have used was almost independent of *p*H within a given range of 0·1 *p*H unit, and readings of virtually instantaneous *p*H change within the given range were proportional to the amount of H^+ ion (ΔH^+) effectively added to or removed from the phase in acid-base equilibrium with the mitochondrial suspension medium (henceforth called the 'outer phase'). It was thus possible, by suitable calibrations of ΔpH versus ΔH^+ with standard acid or base, to compute ΔH^+ values from the observed respiration-driven and ATPase-driven ΔpH values. As we shall show later, there are sound reasons for concluding that the ΔH^+ values obtained in this way represent the protons translocated ($\rightarrow H^+$) across the mitochondrial coupling membrane that separates an inner mitochondrial phase (probably the interior of the cristae) from the outer phase.

For the measurements of $\Delta H^+/O$, the mitochondria were liberated from male Wistar rat livers by homogenization in ice-cold 0·25 M sucrose, and the mitochondria were largely freed of cell debris, microsomes, and 'fluffy layer' by conventional differential centrifugation in 0·25 M sucrose at 4° C. For the measurements of $P/\Delta H^+$, the mitochondria were prepared by a similar procedure, except that a solution containing 0·21 M sucrose, 1 mM potassium chloride, and 10 mM sodium ethylenediamine tetraacetate (EDTA) was substituted for 0·25 M sucrose in order to reduce the concentration of free Mg^{++}. The stock mitochondrial suspensions (60 mg protein/ml.) were stored briefly in 0·25 M sucrose under semi-anaerobic conditions at 4° C before use.

The ΔH^+ measurements were done in a thermostatically controlled cylindrical glass cell of 6 ml. capacity containing an H^+-sensitive glass electrode, a saturated potassium chloride junction leading to a calomel half-cell, a Clark oxygen electrode, and a magnetically driven glass impeller giving vigorous stirring. The cell could be closed by a glass piston, but a shallow groove in the piston, parallel to the axis of the cell, permitted the insertion of a fine glass needle so that nitrogen could be bubbled in to flush oxygen from the cell, and reagents could be added from calibrated micro syringes. Oxygen from the air was excluded by a jet of nitrogen continuously directed at the top of the piston. The normal procedure was to flush the oxygen from the suspension medium with oxygen-free nitrogen while the glass piston was at the top of the cell. The cold mitochondrial suspension (0·6 ml.) was then introduced to give a total volume of 6 ml., the piston was lowered on to the surface of the suspension, and 20 min was allowed for temperature and ionic equilibration before commencement of ΔpH measurements. The H^+-sensitive glass electrode and the potassium chloride junction/calomel half-cell reference system were connected through conventional valve-potentiometer circuitry, the output of which drove a strip-chart recorder giving a full-scale deflexion corresponding to 0·10 *p*H unit. The measurements were routinely made between *p*H 7·0 and 7·2 at 25° C in a medium containing 25 mM sucrose, 150 mM potassium chloride, and 3·3 mM glycyl–glycine–potassium hydroxide buffer, and any variations of these conditions or additions to or changes in this 'normal medium' are referred to in the text. The system generally exhibited a small constant rate of *p*H drift after equilibration, and the time course of ΔpH was corrected for the drift by plotting from a base line having the slope of the *p*H drift. Oxygen was added as air-saturated or oxygen-saturated carbon-dioxide-free 0·15 M potassium chloride from a micro-syringe (injection time < 1 sec), and the amounts of oxygen were computed from the relevant temperature–solubility tables for pure water[10], corrected by a factor of 0·9 for the effect of ionic strength[11]. Mitochondrial protein was estimated by the method of Itzhaki and Gill[12].

Electron transport system. Fig. 1*a* shows a typical time course of ΔpH and ΔH^+ resulting from the oxidation of β-hydroxybutyrate at 25° C. There was a rapid acidification of the suspension medium, beginning immediately after the addition of oxygen, and a slower, approximately exponential decay of ΔpH to zero. The value of $\Delta H^+/O$ corresponding to the peak of the curve of Fig. 1*a* is 5·45. The controlled and uncontrolled rates of oxygen reduction by the mitochondria under these conditions were found to be respectively 10 and 25 μg atom O/g protein min, and thus it can be estimated that in the experiment of Fig. 1*a* the oxygen would be consumed within 5 sec. The $\Delta H^+/O$ quotient for the experiment of Fig. 1*a* was accordingly corrected for ΔpH decay by a small (linear) extrapolation to time 5 sec, giving a value of 5·96. Sixteen measurements of the corrected $\Delta H^+/O$ quotient on four different batches of mitochondria have yielded a mean value of 5·90 ± 0·33. When the amount of oxygen injected into the 6-ml. mitochondrial suspension is varied between 10 and 50 mμg atom O there is little effect on the appropriately corrected $\Delta H^+/O$ quotient, but for larger amounts of oxygen the $\Delta H^+/O$ quotient falls to relatively low values, becoming zero in the steady state. Similar measurements on the $\Delta H^+/O$ quotient for oxidation of 2 mM succinate at 25° C in presence of 0·4 μM rotenone (to stop NAD-linked oxidations) have given a mean corrected value of 3·25 ± 0·08. However, as shown in the typical pair of experiments described by Fig. 2, succinate causes an enhanced ΔpH decay rate (probably due to specific succinate/OH^- antiport), which can be largely suppressed at 5° C. Our measurements of the corrected $\Delta H^+/O$ quotient for succinate oxidation at 5° C give a mean value of 4·07 ± 0·11. Unlike the value for succinate oxidation there is no increase in the $\Delta H^+/O$ quotient for β-hydroxybutyrate oxidation on lowering the temperature from 25° to 5° C, and this is consistent with the fact that the rate of ΔpH decay is not appreciably temperature dependent in the presence of β-hydroxybutyrate.

The $\Delta H^+/O$ quotient for succinate oxidation at 5° C is depressed towards zero as the amount of oxygen is increased, just as in the case of β-hydroxybutyrate oxida-

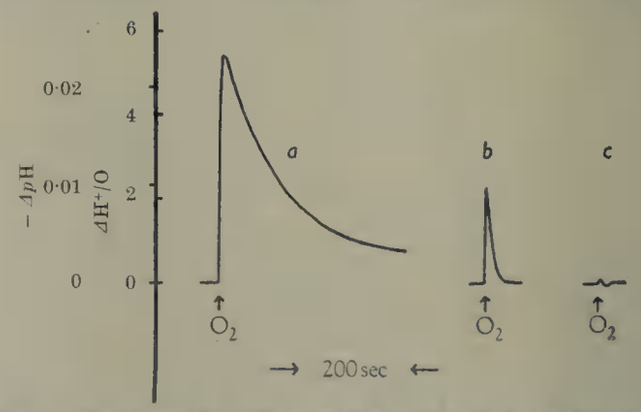

Fig. 1. Time course of ΔpH and ΔH^+ during oxidation of β-hydroxybutyrate at 25° C. Oxygen (23·5 mμg atom in 50 μl. 0·15 M potassium chloride) injected into 6 ml. anaerobic mitochondrial suspension in 'normal medium' containing 2 mM β-hydroxybutyrate at vertical arrows. Curve *a*, no addition; curve *b*, plus 5×10^{-5} M 2 : 4-dinitrophenol; curve *c*, plus 'Triton *X*-100' (1 mg/ml.)

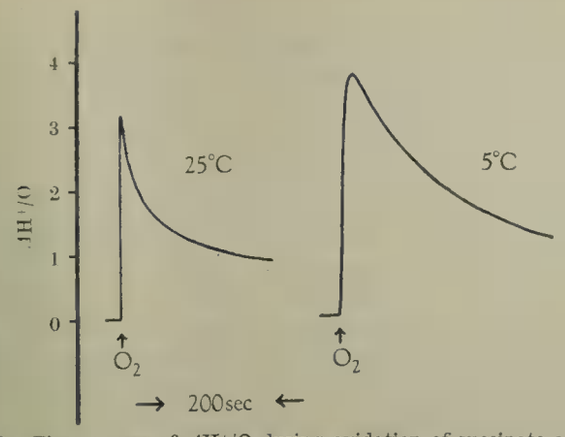

Fig. 2. Time course of $\Delta H^+/O$ during oxidation of succinate at 25° C and at 5° C. Oxygen (23·5 mμg atom in 50 μl. 0·15 M potassium chloride) injected into 6 ml. anaerobic mitochondrial suspension in 'normal medium' containing 2 mM succinate plus 0·4 μM rotenone at vertical arrows

tion. The presence of 6 μg oligomycin per ml. mitochondrial suspension enhanced the $\Delta H^+/O$ values for both β-hydroxybutyrate and succinate by 0–8 per cent in different experiments. Our results suggest that, ideally, the $\Delta H^+/O$ quotients for succinate and β-hydroxybutyrate oxidation are respectively 4 and 6.

We have interpreted the observed acidification of the suspension medium in the experiments described here as the consequence of a stoichiometric net translocation of protons through the mitochondrial coupling membrane. In order, however, to justify this interpretation, we must exclude the possibility that the observed $\Delta H^+/O$ values might be due to the stoichiometric accumulation of an anhydride[13] (for example, $X \sim I$) the synthesis of which would involve the net dissociation of $2H^+$ ions per ~ bond. Fig. 1*b* shows that when 5×10^{-5} M 2 : 4-dinitrophenol is present, in an experiment otherwise identical to that of Fig. 1*a*, there is a much faster ΔpH decay, and the height of the ΔH^+ peak is reduced. Similarly, as shown in Fig. 1*c*, 'Triton *X*-100' (1 mg/ml.) in place of dinitrophenol, virtually abolishes ΔpH and ΔH^+. Other uncoupling and membrane-lytic agents have been found to behave similarly to DNP and 'Triton *X*-100' respectively in this type of experiment. These observations suggest that the respiration-driven pH displacement and its subsequent decay are due to the effective translocation of H^+ or OH^- ions across the coupling membrane and their subsequent re-equilibration by diffusion or exchange through the membrane, for dinitrophenol and other uncouplers are known to catalyse the equilibration of H^+ ions across the mitochondrial membrane system[3,14] and the membrane-lytic agents permit general ionic equilibration between the phases originally separated by the membrane. However, these observations do not completely eliminate the aforementioned possibility that the respiration-driven pH displacement and its subsequent decay might be due to the formation of an $X \sim I$ anhydride in a lipid phase, the subsequent hydrolysis of $X \sim I$ being catalysed by uncouplers and by the detergent membrane-lytic reagents. A different experimental approach is required to shed more light on this question.

Fig. 3 shows a group of four observations under conditions identical to those of Fig. 1*a*, except that in all but the control (*A*) stoichiometric amounts of potassium hydroxide were present in the air-saturated saline injected into the mitochondrial suspension. We have observed in a number of such experiments that, after the respiration-driven pH displacement: (i) the rapid pH shift caused by the potassium hydroxide present in the saline is proportional to the amount of potassium hydroxide added and is not evidently enhanced or decreased by any chemical reaction consequent on changing the pH with the potassium hydroxide solution; (ii) the subsequent change of the pH of the outer medium with time can be either positive or negative, depending on the outer pH, within a total range of only 0·15 pH unit; (iii) there is one particular pH at which the outer medium will remain constant after the respiration-driven pH displacement.

If the initial respiration-driven acidification of the outer medium were due to the formation of an $X \sim I$ anhydride, and the subsequent hydrolysis of $X \sim I$ were responsible for the ΔpH decay, it is difficult to account for the fact that the upward pH movement corresponding to the ΔpH decay is eliminated by raising the outer pH by only 0·1 unit relative to the initial pH, and that it is actually reversed by raising the outer pH by 0·2 unit—especially as the normal $\Delta H^+/O$ values have been found in other experiments to be almost independent of pH between pH 6 and 8. On the other hand, if there is no net production or consumption of H^+ ions during the respiration-driven pH displacement, but simply an effective displacement of H^+ ions outwards (or OH^- inwards) across the coupling membrane, the fall in the external pH ($-\Delta p\mathrm{H}_O$) should be accompanied by a rise in the internal pH ($\Delta p\mathrm{H}_I$), and the magnitudes of $\Delta p\mathrm{H}_I$ and $-\Delta p\mathrm{H}_O$ should be in the inverse ratio of the buffering powers ($\Delta H^+/\Delta p$H) of the respective phases. Further, the $\Delta p\mathrm{H}_O$ decay, being due to the inward diffusion or exchange of H^+ ions (or outward diffusion or exchange of OH^- ions), should depend on the pH differential across the membrane, and the $\Delta p\mathrm{H}_O$ decay should therefore be arrested or reversed by raising the external pH ($p\mathrm{H}_O$) appropriately. Assuming that the pH differential across the membrane ($p\mathrm{H}_I - p\mathrm{H}_O$, or $p\mathrm{H}_I + \Delta p\mathrm{H}_I - \{p\mathrm{H}_O + \Delta p\mathrm{H}_O\}$) tends to decay to zero or to some constant value, the internal respiration-driven pH displacement will be given by the total shift of the outer pH required to result in a stable outer pH value after the respiration-driven H^+ displacement. It will be seen from Fig. 3 that the respiration-driven $\Delta p\mathrm{H}_O$ is 0·0120 unit, giving a corrected value of 0·0135 unit, and that $\Delta p\mathrm{H}_I$, equated with the total $\Delta p\mathrm{H}_O$ required to give a stable pH after the respiration-driven H^+ displacement, is approximately 0·11 pH unit. The ratio $\Delta p\mathrm{H}_I/\Delta p\mathrm{H}_O$ due to the respiration-driven H^+ displacement is thus about 8. It has been possible to estimate the buffering powers of the inner and outer phases over the appropriate pH range by titrating mitochondrial suspensions with standard hydrochloric acid or potassium hydroxide, (*a*) adding aliquots of acid or alkali quickly and extrapolating the titre to zero time, giving the buffering power of the outer phase ($\Delta H_O^+/\Delta p\mathrm{H}_O$); (*b*) titrating in presence of reagents catalysing equilibration of H^+ across the membrane (for example, 5×10^{-5} M DNP + 200 μg

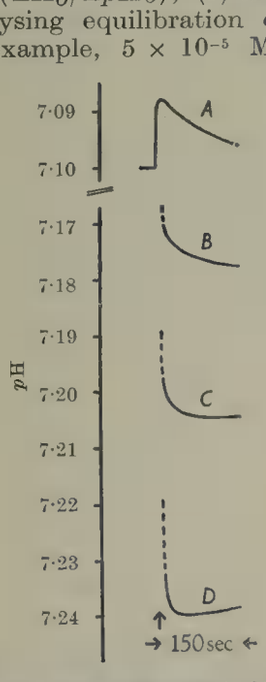

Fig. 3. Effect of injection (at vertical arrows) of 25 μl. 0·15 M potassium chloride containing oxygen (11·8 mμg atom) and different amounts of potassium hydroxide on the pH of an anaerobic mitochondrial suspension in the presence of β-hydroxybutyrate at 25° C. *A*, No potassium hydroxide; *B*, plus 475 mμ equiv. potassium hydroxide; *C*, plus 665 mμ equiv. potassium hydroxide; *D*, plus 855 mμ equiv. potassium hydroxide. External buffering power of the mitochondrial suspension across pH 7·15 in this experiment is 5·7 μequiv./pH unit

'Triton X-100' per ml.) to obtain the sum of inner and outer buffering powers ($\Delta H_O^+/\Delta pH_O + \Delta H_I/\Delta pH_I$). The buffering power of the inner phase ($\Delta H_I^+/\Delta pH_I$) is given by difference. For the mitochondria used in these experiments in the 'normal medium' the approximate values of $\Delta H_I^+/\Delta pH_I$ and $\Delta H_O^+/\Delta pH_O$ are 130 and 950 mμ equiv. H^+/pH unit ml. mitochondrial suspension (containing 6 mg protein/ml.) respectively across pH 7·15, giving a buffering power ratio of 7·3. This agrees satisfactorily with the $\Delta pH_I/\Delta pH_O$ of 8 estimated above.

There appear from the foregoing experiments to be rather convincing grounds, both qualitative and quantitative, for concluding that the respiration-driven pH changes described in this article are caused by the effective stoichiometric displacement of H^+ ions (or OH^- ions) across the coupling membrane of the mitochondria, and that the $\Delta pH/O$ and $\Delta H^+/O$ values can be properly used to derive the $\rightarrow H^+/O$ quotients.

ATPase system. Fig. 4*a* shows a typical time course of ΔpH and ΔH^+ on injecting 0·1 μmole ATP in 10 μl. 0·15 M oxygen-free potassium chloride into 6 ml. mitochondrial suspension (6 mg protein/ml.) in 'normal medium' containing 1·0 mM EDTA, but no substrate, at 25° C. Under these conditions the activity of the adenylate kinase is very low[11]. There is a fairly rapid acidification of the suspension medium, followed by a slower, approximately exponential decay of ΔpH towards a value corresponding to $\Delta H^+/P = 0·8$. Experiments such as that of Fig. 4*b*, in which 6 μg oligomycin per ml. plus 5×10^{-5} M DNP were present, confirm that the final value of $\Delta H^+/P$ represents the acidification due to the chemical reaction $ATP + H_2O \rightarrow ADP + Pi + (0·76–0·80)H^+$ at pH 7·0–7·1. The uncorrected height of the peak in the experiments of Fig. 4*a* represents a total $\Delta H^+/P$ quotient of 2·08, or a quotient of 1·30 on subtracting the base-line value of 0·78. Extrapolation to correct for ΔpH decay is more difficult in this type of experiment than in the oxido-reduction experiments because the rate of acidification is considerably slower initially and decreases as the ATP concentration falls during hydrolysis. We adopted the empirical method of extrapolating to the point of intersection of the straight lines representing the initial rate of pH fall and the initial straight portion of the subsequent ΔpH decay. The value is thus corrected to 1·49 in the experiment of Fig. 4. The extent of the side reactions for ATP hydrolysis was determined by the fraction of the overall rate that is oligomycin (6 μg/ml.) insensitive. In the typical mitochondrial preparation used for the experiment of Fig. 4 the oligomycin-insensitive ATPase represented 22 per cent of the total ATPase, and, accordingly, the corrected value of the $\Delta H^+/P$ quotient is 1·91. Several groups of similar experiments on four different batches of mitochondria have given a mean corrected $\Delta H^+/P$ quotient of 1·88 ± 0·09. Conversely to the $\Delta H^+/O$ quotients, the $\Delta H^+/P$ quotients have been found to be suppressed by oligomycin (6 μg/ml.), but unaffected or slightly enhanced by antimycin A (0·12 μg/ml.) or by rotenone (0·4 μM). As described for the case of the electron transport system, by shifting the pH upward as the peak of ΔpH is reached during ATP hydrolysis, the pH changes in the suspension medium caused by the ATPase system can be shown to be due to an effective displacement of H^+ ions through the coupling membrane and not to the net production or absorption of H^+ ions during the synthesis or breakdown of a hypothetical $X \sim I$ compound.

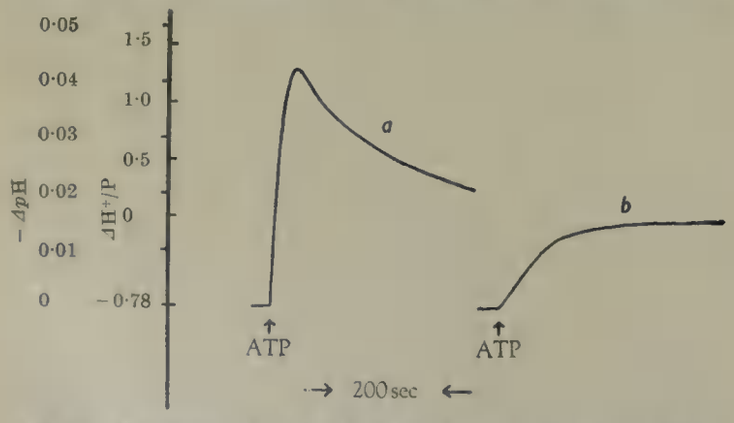

Fig. 4. Time course of ΔpH and $\Delta H^+/P$ during hydrolysis of ATP at 25° C. ATP (0·1 μmole in 10 μl. 0·15 M oxygen-free potassium chloride) injected into 6 ml. anaerobic mitochondrial suspension in 'normal medium' containing 1·0 mM EDTA at vertical arrows. Curve *a*, no addition; curve *b*, plus 5×10^{-5} M DNP and oligomycin (6 μg/ml.)

Ion secretion and electron translocation. There is a difference of opinion as to whether the 'secretion of H^+ ions' by continuously respiring mitochondria in response to the addition of divalent cations or of valinomycin or gramicidin is a primary process[9], or whether it is a consequence of the 'active' uptake of the cations[8]; and we have therefore made a preliminary examination of this question for the case of the stoichiometric respiration-driven H^+ ion translocation observed in our experiments. Substituting a miniature flowing liquid junction (to be described in detail elsewhere) for the normal saturated potassium chloride junction of the reference electrode system, we have observed that, under appropriate conditions of pH and temperature, the limiting $\Delta H^+/O$ quotient for β-hydroxybutyrate oxidation is not significantly affected by replacing the 0·15 M potassium chloride of the suspension medium by an equal concentration of sodium chloride or choline chloride, or by substituting 0·25 M sucrose for the salt. The presence or absence of 3 mM magnesium chloride in the foregoing media was found to be without effect on the $\Delta H^+/O$ quotient. The K^+ content of the mitochondria in these experiments gave an equilibrium external K^+ concentration of *c.* 5×10^{-4} M; it was observed, by means of a K^+-sensitive glass electrode, that in the sucrose medium containing 3 mM magnesium chloride, and in the choline chloride medium, the respiration-driven outward H^+ displacement was accompanied by an uptake of K^+ ions. However, the K^+ ion uptake commenced slightly later than the H^+ displacement, was much less in magnitude than the H^+ displacement, and the subsequent K^+ extrusion was complete or showed a significant reversal about half-way through the time course of the ΔpH decay. We conclude that the respiration-driven H^+ translocation is probably an integral consequence of the passage of electrons and hydrogen atoms in opposite directions through the vectorially organized respiratory chain system of the mitochondrial membrane; and in this sense the respiration-driven displacement of H^+ or acid equivalents across the coupling membrane can probably be classed as a "primary secretion of H^+ ions".

In the case of the ATPase-driven H^+ translocation, it also seems probable, as will be described elsewhere, that cyclic transitions of mobile chemical groups through different states of ionization and hydration give rise to the passage of electrons and hydrogen atoms across the ATPase system in opposite directions.

Summarizing the experimental findings: (1) Oxido-reduction through the electron-transport chain and ATP hydrolysis through the ATPase system each results in the translocation of protons outwards through the mitochondrial membrane system. (2) The stoichiometry of the ATPase system closely approximates to a $P/\rightarrow H^+$ quotient of 1/2, while the $\rightarrow H^+/O$ quotients for succinate and β-hydroxybutyrate oxidation approximate closely to 4 and 6 respectively; thus making the product of the $P/\rightarrow H^+$ and $\rightarrow H^+/O$ quotients for succinate and β-hydroxybutyrate close to the known P/O quotients of 2 and 3.

These observations are in close accord with the basic principles of the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation, but show that the following changes of detail are required in Mitchell's original formulation[1]: (i) As already surmised[2], the polarity of the system corresponds to a lower electrochemical potential of H^+ inside than outside the coupling membrane (higher pH,

more negative electric potential), the reverse of that originally assumed. (ii) The $\rightarrow H^+/O$ and $\rightarrow H^+/P$ stoichiometries are double those originally assumed. (iii) Owing to (ii), the electrochemical potential differential of H^+ ions across the coupling membrane required to cause a given poising effect on the oxido-reduction and ATPase equilibria is only half that previously estimated; that is, a differential of 3·5 *p*H units with no membrane potential or a membrane potential of 210 mV with no *p*H differential would be sufficient to poise the ATP/ADP quotient central at a P*i* concentration of 10 mM.

The latter point is particularly helpful to the chemiosmotic interpretation of the coupling mechanism because it brings the *p*H and electric potential differentials required by the hypothesis within the physiological limits usually accepted for these variables (see Mitchell[4]). As shown elsewhere, the chemiosmotic hypothesis is now able to provide as realistic a model of oxidative phosphorylation as the more fashionable chemical hypothesis, and in particular it affords a very simple explanation of the action of classical uncoupling agents such as dinitrophenol, of mitochondrial swelling phenomena, of the uptake and uncoupling effects of polyvalent metal ions, of the special mechanism of uncoupling by valinomycin and gramicidin, and of the pseudo site-specific inhibition of respiration by organic cations such as guanidine and its derivatives[4]. Fuller accounts of the work presented in this article and a separate account of the present status of the theory of chemiosmotic coupling will be published in due course.

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STRUCTURE OF CONDENSED TANNINS

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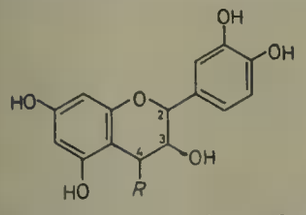
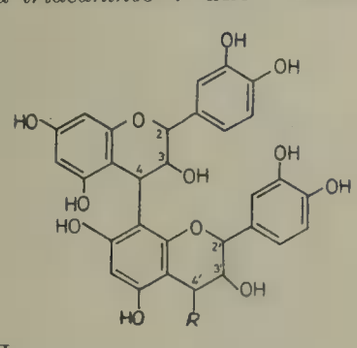
MOST of the work that has so far been carried out on the structure of condensed tannins (flavolans) has been concentrated on the possible modes of polymerization of the hydroxyflavan-3-ols (for example, (+) catechin, I*a*)[1–5]. The polymerization of hydroxyflavan-3,4-diols (for example, II*a*,*b*), on the other hand, has been less completely investigated, although it is believed that they are intimately concerned in the formation of flavolans[6–8], and model experiments have shown that the hydroxyl group at C-4 readily forms carbonium ions in acid solution (for example, III) which react with nucleophilic centres similar to those at C-6 and C-8 in the commonly occurring flavan-3-ols and -3,4-diols (for example, I*a*, I*b*, II*a* and II*b*)[8–10]. Indeed, several workers[2,11,12] have suggested that the flavolans are formed from flavan-3,4-diols by linkages between C-4 of one unit and C-8 (ref. 13) (or C-6, ref. 9) of another (for example, IV). Freudenberg[2] has further postulated that such structures would be readily cleaved by acid treatment to yield the parent diols (for example, II) or its reaction products (for example, the corresponding anthocyanidin, VI).



I: R=H
a, 2,3-*trans* (+)-catechin
b, 2,3-*cis* (−)-epicatechin
II: R=OH
a, 3,4-*trans*
b, 3,4-*cis*
III: R= ⊕
(Carbonium ion)

In spite of this hypothesis, it has been suggested that the naturally occurring flavan dimers (biflavans) which have so far been investigated[14–16], and which yield on treatment with dilute acid under relatively mild conditions (for example, 0·1 N HCl for 5 min at 100°)[14] a flavan-3-ol (either I*a*[16] or I*b*[14,15]) and a second flavan moiety (which in all cases[14–16] gives cyanidin (VI) on heating in stronger acid), have structures involving either a hemiacetal[14], ether[15,17] or hemiketal[16] linkage between the hydroxyl group at C-3 of the flavan-3-ol and one of the hydroxyl groups of the pro-anthocyanidin moiety. Geissman and Dittmar[18,19], on the other hand, have adduced evidence that the biflavan from avocado seed (*Persea gratissima*), which yields catechin (I*a*) on mild acid hydrolysis, has the structure V*a*. They have further suggested that the most commonly occurring polymeric leucoanthocyanins (that is, flavolans which yield cyanidin on acid treatment) are formed by extensive self-condensation of (III) via (IV), but that when a flavan-3-ol (for example, catechin (I*a*)) is present a biflavan can be produced (for example, V*a–e*) which is relatively stable and is incapable of further condensation since it lacks a hydroxyl group at C-4′ (ref. 1).

We have been studying the physiology of flavolan formation in the leaves of the wild strawberry (*Fragaria vesca*) and have shown that this tissue produces, besides (+)-catechin (I*a*) and a number of flavolans of apparently increasing complexity, a biflavan which yields (+)-catechin (I*a*) and cyanidin (V) by suitable acid treatment, and is thus similar to the biflavans from avocado[18,19] and *Gleditschia triacanthos*[16]. The λ max and molecular



IV: R=OH
V: R=H
a, Stereochemistry at C-2, -3 and -4 undetermined 2′,3′-*trans*
b, 2,3-*trans*-3,4-*trans*-2′,3′-*trans*
c, 2,3-*trans*-3,4-*cis*-2′,3′-*trans*
d, 2,3-*trans*-3,4-*trans*-2′,3′-*cis*
e, 2,3-*trans*-3,4-*cis*-2′,3′-*cis*

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absorptivity of the solutions of the biflavans from strawberry and avocado, both in methanol and 70 per cent sulphuric acid[20], and of their adduct with vanillin in 70 per cent sulphuric acid[21] are closely similar, as is the yield of cyanidin formed by heating either of them in *n*-butanol-concentrated hydrochloric acid (9 : 1)[21] for 1 h (Table 1). However, the R_F values of the two compounds are different in most solvent systems and it must be assumed that the two compounds are stereoisomers. Support for this assumption comes from the fact that, after epimerization in water at 120° for 2 h[22], the strawberry biflavan gives six components separable on cellulose thin-layer plates in 5 per cent methanol, three of which have identical R_F values with the products of epimerization of the avocado compound.

In order to obtain more evidence as to the nature of the strawberry biflavan, we have examined the products obtained by treating an aqueous solution of a mixture of (+)-catechin (I*a*) and the flavan-3,4-diol racemate (II*a*,*b*) produced by the reduction of naturally occurring 2,3-dihydroquercetin (2,3-*trans*) with sodium borohydride[23], with cold 0·4 *N* hydrochloric acid for 5 min. The acid was removed with di-*N*-octyl methylamine in chloroform[24], and the resultant mixture separated by preparative paper-chromatography in butanol/acetic acid/water (BAW, Table 1), giving two compounds *CA* and *CB* (see Table 1) in yields of 50 per cent and 37 per cent respectively (based on the (+)-catechin used). The R_F value of the faster-running major component *CA* on paper chromatograms and on three different thin-layer supports in various suitable solvent systems is identical with that of the strawberry biflavan (Table 1). Furthermore, both *CA* and *CB* have the same spectral properties as the strawberry compound (Table 1) and produce the same six components on epimerization[22]. It should be noted that both *CA* and *CB* give (+)-catechin (I*a*) on treatment with dilute acid and are presumably stereoisomers at C-4 (V*b* and V*c*). From the chromatographic behaviour of the known isomeric flavan-3,4-diols[22], and the fact that sodium borohydride reduction of the dihydroflavonols gives mainly 3,4-*trans* compounds[25], it appears possible that the major synthetic dimer, and therefore the strawberry biflavan, is the 2,3-*trans*-3,4-*trans*-2′,3′-*trans* isomer (V*b*); *CB* therefore being V*c*. Naturally, more exact criteria[22,25,26] are required to confirm this possibility.

When (−)-epicatechin (I*b*) was mixed with the flavan-3,4-diol racemate (II*a*,*b*) and treated as above, two different compounds were produced, *EA* and *EB* (50 per cent and 22 per cent yield of each based on the (−)-epicatechin). With suitable acid treatments, these compounds gave (−)-epicatechin (I*b*) and cyanidin (V) and both had almost identical spectral properties as those of the biflavan isolated from the seeds of *Theobroma cacao*[27]. However, the R_F values of both synthetic compounds are different from that of the natural product. Epimerization of both *EA* and *EB* gives six separable products (two of which are identical in R_F value with two of those produced from *CA* or *CB*, but the cocoa biflavan only yields three separable components, one of which corresponds with *EA* while another corresponds with *CA*. Since *EA* and *EB* must be IV*d* and IV*e*, it appears probable that the cocoa biflavan may differ in the stereochemistry at C-2 and C-3 (V), and is perhaps the 2,3-*cis* isomer. If this were the case both flavan moieties would have the same configuration at C-2 and C-3. It is, of course, possible that some other structure is involved, but since the octamethyl ether of the cocoa compound yields 5,7,3′,4′-tetra-*O*-methyl-epicatechin and 5,7,3,4′-tetra-*O*-methylcyanidin on treatment with hot acid[14] it must be concluded that the phenolic hydroxyl groups are not involved in the linkage between the flavan nuclei, and structures such as those suggested by Roux[11] and Hergert[12] for the formation of polymers involving a link between C-4 and the oxygen at C-7 of the second flavan are unlikely.

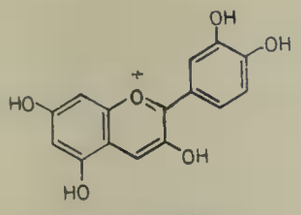
Finally we have examined the properties of the water-insoluble, methanol-soluble polymer *LP* which is produced when the flavan-3,4-diol (II*a*,*b*) is treated with cold acid[13,23] in the absence of any other nucleophile. This compound is immobile in all solvent systems and shows the expected lower reactivity with vanillin in 70 per cent sulphuric acid[21,28], but gives a somewhat higher yield of cyanidin than found with natural polymers of this type[7,13] (Table 1). It also yields no (+)-catechin or (−)-epicatechin on mild hydrolysis: when the hydrolysis is carried out in the presence of (+)-catechin, however, it does give small yields of both *CA* and *CB*, thus indicating that the C–C links are broken by such treatment. Since Quesnel[29] has shown that the natural cocoa flavolan yields (−)-epicatechin on mild acid hydrolysis, it must be concluded that Geissman and Dittmar's hypothesis with regard to the structure of the flavolans is not wholly correct.

It appears probable, instead, that condensed tannins of the flavolan type are complex, branched, three-dimensional structures in which the majority of the repeating units are nuclei derived from hydroxyflavan-3-ols and -3,4-diols. It is also possible that nuclei derived from flavan-4-ols and flaven-4-ols[30] (flavyllium pseudobases) may be involved. It is considered that the flavan nuclei will be linked, initially, mainly by bonds between C-4 of one of the flavan-3,4-diol units, and either of the nucleophilic sites, C-8 (ref. 13) or C-6 (especially the latter for those hydroxy flavans having a resorcinol-like *A*-ring[9], for example, VII) of another hydroxyflavan. In the case of flavans having a pyrogallol-type of substitution in the *B*-ring (for example, VIII) there will also be the possibility of links to the nucleophilic C-2′ and C-6′ positions (cf. refs. 3 and 4). In such polymers flavan-3-ol nuclei, linked via C-4 of a flavan-3,4-diol, would form relatively stable end units to branches, and the parent flavan-3-ol would thus be released on mild acid treatment[29]; similarly,

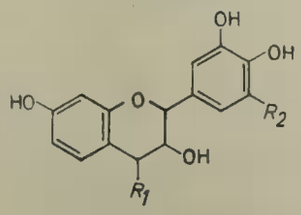
Table 1. Properties of Natural and Synthetic Biflavans

Compound	R_F on thin-layer plates*				$\varepsilon \times 10^{-3}$			Yield of cyanidin (molar %)	Products of mild hydrolysis
	Cellulose			Silica gel-H Bz : Ac : W** 10 : 10 : 1	MeOH†	70% H_2SO_4‡	1% Vanillin in 70% H_2SO_4§		
	BAW 4 : 1 : 2·2	5% aq. MeOH	80% phenol						
Avocado biflavan	0·47	0·23	0·10	0·15	6·2	9·0	27·6	38	Epicatechin and catechin
Strawberry biflavan	0·45	0·30	0·13	0·31	6·6	8·4	24·5	35	Catechin
Cocoa biflavan	0·55	0·50	0·19	0·31	6·0	8·8	29·5	30	Epicatechin
CA	0·45	0·30	0·13	0·31	6·3	8·7	20·8	28	Catechin
CB	0·35	0·28	0·06	0·12	††	–	–	–	Catechin
EA	0·52	0·39	0·21	0·31	5·7	10·0	33·0	28	Epicatechin
EB	0·40	0·25	0·09	0·13	††	–	–	–	Epicatechin
(+) Catechin	0·68	0·33	0·37	0·79	3·6	8·7	30·1	–	–
(−) Epicatechin	0·54	0·31	0·38	0·72	3·6	10·8	35·6	–	–
Leucocyanidin	0·57	0·41		0·64	3·2	3·9	15·8	40	–
LP	0	0	0	0	4·8¶	6·1¶	13·9¶	17¶	–

* Thin-layer chromatography on polyamide in 70 per cent aq. MeOH; all biflavans had *R* 0·63, monomers 0·50, and *LP* 0. Solvents: BAW, *n*-butanol-acetic acid-water. Bz : Ac : W, benzene-acetic acid-water. ** Developed twice. † At 280 mμ (λmax. in each case). ‡ At 355 mμ (λmax. in each case). § At 500 mμ. ††The ε values for both *CB* and *EB* in MeOH were ~20 per cent lower than *CA* and *EA* respectively, perhaps due to the presence of interfering materials which were not resolved chromatographically: the relative absorptivities in MeOH, 70 per cent H_2SO_4 and with vanillin in 70 per cent H_2SO_4 and the molar yield of cyanidin, however, were more or less the same for *CA*, *CB* and *EB*. ¶ Based on monomer.



VI: Cyanidin



VII: R_1 = H or OH
R_2 = H
VIII: R_1 = H or OH
R_2 = OH

flavan-3,4-diol nuclei present in the structure linked as end units, or only through other diols, would also be released on acid treatment to give in part anthocyanidins. Branch points would occur wherever two or more of the potential reactive centres in any one flavan were involved in linkage. Subsequent changes in the initial polymer may be induced by oxidation[3,4] (which might be better regarded as being due to one-electron coupling reactions; compare refs. 31, 32), by the formation of bonds between the electrophilic centre at C-2[1,2] and the nucleophilic sites mentioned earlier, and by the formation of internal ether-type links[30,33,34]. Of course, other suitable nucleophiles or electrophiles (such as lignin precursors, amino-acids, etc.) may also be incorporated into the whole flavolan molecule, and links may be formed to other macromolecules in the cell[28].

In spite of our doubts about Giessman and Dittmar's proposals[18,19] for the flavolans, we believe that the results presented here support their hypothesis regarding the structure and stability of the biflavans. Furthermore, this theory may be extended to explain why the only natural flavan-3,4-diols so far isolated which have had their structure and stereochemistry unequivocally proven all lack a hydroxyl group at C-5 (for example, VII, R_1 = OH). The lack of this hydroxyl group not only enhances the stability of the flavan-3,4-diol itself by reducing the nucleophilic activity of C-6 and C-8, but also reduces the probability of its reacting with a corresponding flavan-3-ol. It is noteworthy that in spite of extensive investigations[8] no biflavans corresponding to IV having a resorcinol-type substitution in either or both *A* rings have so far been isolated.

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INTERSTIMULUS INTERVAL AND NUMBER JUDGMENT

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ON the basis of previous experiments it was suggested by one of us that the interference which occurs when subjects respond to two single stimuli, separated by very short time-intervals, was determined by both perceptual and motor components[1]. In a more recent paper it has been shown that some subjects can, under certain conditions, process simple stimuli simultaneously and independently[2]. In the experiments reported here we examined some aspects of the perceptual field which may determine the effective size of the so-called span of apprehension. The size of the span of visual apprehension is a subject which has attracted a fair amount of psychological investigation. Less work, however, has been done on the problem of the necessary length of the time-interval needed between two stimuli to allow two independent judgments, or on the interaction between this interval and the content of the two stimuli. The many investigations into the span of visual apprehension all agree that the accuracy of judging the number of briefly displayed objects decreases with increasing stimulus number, and that, under most conditions of presentation, the threshold criterion of 50 per cent correct responses occurs with 6–7 stimulus objects. Three experiments on the effect of interstimulus interval on the perception of numerosity are described.

In the first experiment 6 subjects were asked to report the number of neon bulbs illuminated in two presentations, separated by time-intervals of 50, 100, 200, 350, 600 and 900 msec. Because earlier experimental findings suggested that some subjects could process simple stimuli independently if they were presented in the opposite half-fields, the display was designed so that pairs of stimuli could be presented in the same half-field or in different half-fields.

Sixty-four small neon bulbs were mounted behind small apertures in a black display board in the form of four small 4 in. × 4 in. square arrays which formed the corners of a square of side 1 ft. At the centre of the display board there was a small warning light which the subject was instructed to use as a fixation point. The warning light

came on 1,200 msec before the start of each stimulus situation and remained on for 800 msec.

The pairs of stimuli were arranged in sets of 36, each set consisting of all combinations of 1–6 lights in the first stimulus, with 1–6 lights in the second stimulus. The number sequences, the spatial relationships and the time separation of the stimuli were determined by a balanced, randomized design. Each subject was tested twice, each session consisting of 6 sets of stimuli, separated by a rest interval of 2 min. The subjects were 6 female medical students at the Royal Free Hospital, their mean age being 24 years. The subject's instructions were to give a verbal report of the presented number for both stimuli in each situation.

The first of each pair of stimuli could be presented in any of the 4 positions and the second followed it at the selected interval in any of the other 3 positions. The duration of the neon flash was 40 msec and the time intervals were measured from the start of the first stimulus to the start of the second. The subject was seated 8 ft. from the display; the visual angle sub-tended by the distance between the warning light and the neon positioned at each outermost corner was 5 deg.

The results of this first experiment are given in Table 1. The findings are disappointing. An analysis of variance showed no significant position effect and that the effect of the value of the time-interval on the number of correct responses was significant only at the 5 per cent level. It was, however, clear from the overall performance of our subjects, and from their introspective reports, that the effects of the variables were obscured by the fact that the task was too difficult under all conditions. It seemed likely that at least three factors were contributing to this. In the first place, the stimulus arrays were outside central vision, making the resolution of each stimulus difficult. This was confirmed in a subsidiary experiment in which the subjects reported the number of neons presented in single groups, in one condition with central fixation and random presentation of the array, and in a second condition in which they were told in which array the stimulus would appear and were allowed to fixate on that position. A second difficulty was that the use of a peripheral stimulus and the unbalance of expectancy, created by the fact that the second stimulus occurred with equal frequency in two contralateral and one unilateral quadrants, made it very difficult for subjects to inhibit eye movements occurring between the tw stimuli. These eye movements were both reflex towards the first stimulus and, if this was inhibited, 'expectant' towards the contralateral half-field. Thirdly, the experimental design did not allow enough practice.

In order to overcome these difficulties, we decided to leave for the time being the examination of the half-field effect and to design an experiment which would provide reliable information on the effect of the time-interval. An alternative display was designed and built.

In the second experiment 60 small neon bulbs were arrayed in 3 concentric hexagonal rings. The outermost hexagon had a radius of 2 in. and was made up of 30 neons which formed 1 functional group. The middle hexagon had a radius of 1·2 in. and contained 18 neons and the inner one, with a radius of 0·8 in., was made up of 12 neons. The 30 neons in the 2 inner hexagons formed the second functional group. As in the first experiment, there was a warning light at the centre of the display.

Table 1

	Interval (msec)						Average for all intervals
	50	100	200	350	600	900	
Stimuli in opposite half-fields	28·1	34·7	28·8	32·2	36·8	36·1	32·8
Stimuli in same half-fields	30·6	26·4	31·9	31·9	35·4	41·0	32·9
Average for all conditions	28·9	31·9	29·9	32·2	36·3	37·7	32·8

Percentage of correct pairs of responses for all 6 subjects in Experiment 1 at each of the time-intervals is given separately for the presentations made in the same and the opposite visual half-fields.

The visual angle sub-tended by the display at the viewing distance of 8 ft. was less than 2·5°.

The stimuli were arranged in basic sets of 36 pairs consisting of all combinations of 1–6 neons in the first stimulus, with 1–6 neons in the second stimulus. The sets of stimuli were presented in the 2 sequences inside followed by outside and outside followed by inside. Each subject was required to judge the presented number for 2 practice and 4 test sets of stimuli, at each of the following 12 intervals: 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 700 and 900 msec.

Each time-condition was examined separately, half the subjects taking the series in ascending order and half in the descending order. The subjects were free to report on the total number of lights for both stimuli or to make separate estimates. The subjects were students or members of staff at the Royal Free Hospital. Eight were female and 4 were male, the mean age for the group being 26 years.

The results of this experiment are summarized in Fig. 1. At intervals of 200 msec and longer, all 12 subjects made separate reports for each member of the stimulus pairs. At the shorter time-intervals most of the subjects made single reports in response to each pair of stimuli. For the analysis presented here the report is regarded as an error when the total number reported was different from the total number presented. In Fig. 1 the results are presented for both the descending and ascending groups. At all intervals, except 300, 700 and 900 msec, the ascending group made more errors than the group taking the time-intervals in descending order.

The differences in the error curves for the 2 groups of subjects strongly support the view that different mechanisms or strategies are employed at the very brief and longer time-intervals. Both groups show evidence of a practice effect at the task of judging stimuli separated by the longer time-intervals, that is, in the range from about 100 to 900 msec. The ascending group performed relatively badly at the first intervals they encounter in this range; but at the longest intervals which are the first encountered by the descending group, the performance levels are reversed. At the simultaneous condition and the 50 msec time-interval the prior experience of the groups of the shorter intervals is about the same and the performance levels are almost identical. Both curves show fewer errors when the stimuli are presented simul-

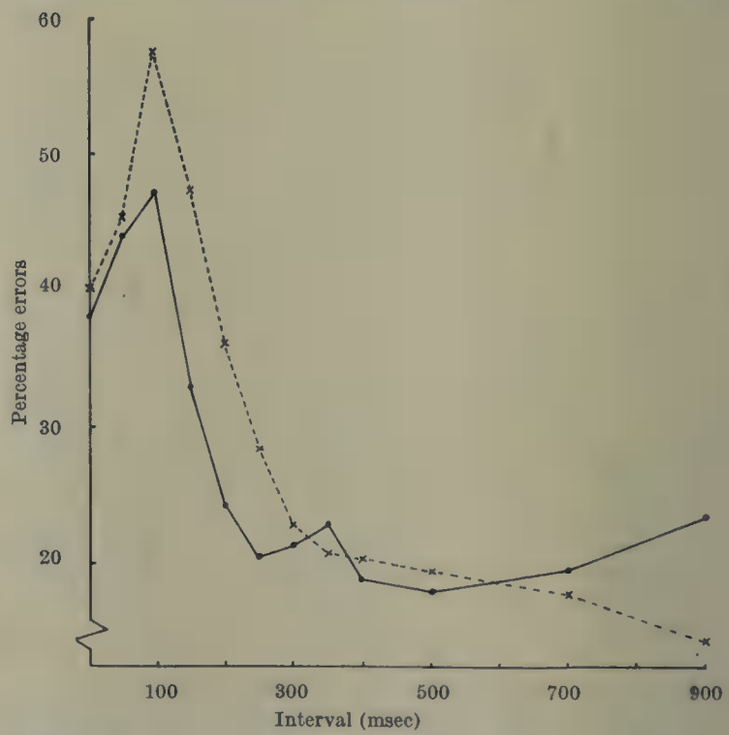

Fig. 1. The error curves obtained in the second experiment for the two groups of 6 subjects—ascending and descending orders of time-intervals. – – –, Group 1 ascending; ——, group 2 descending

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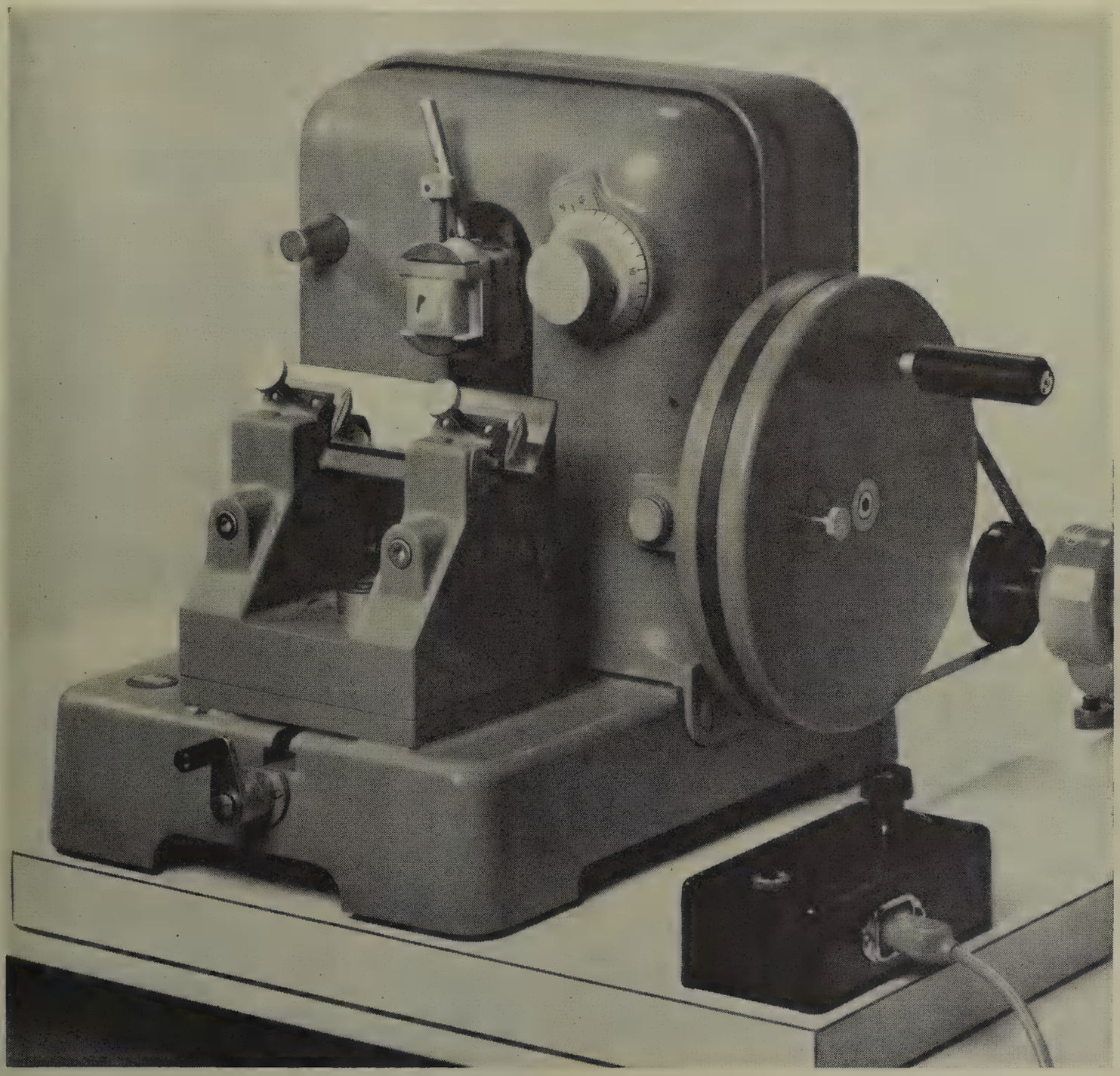

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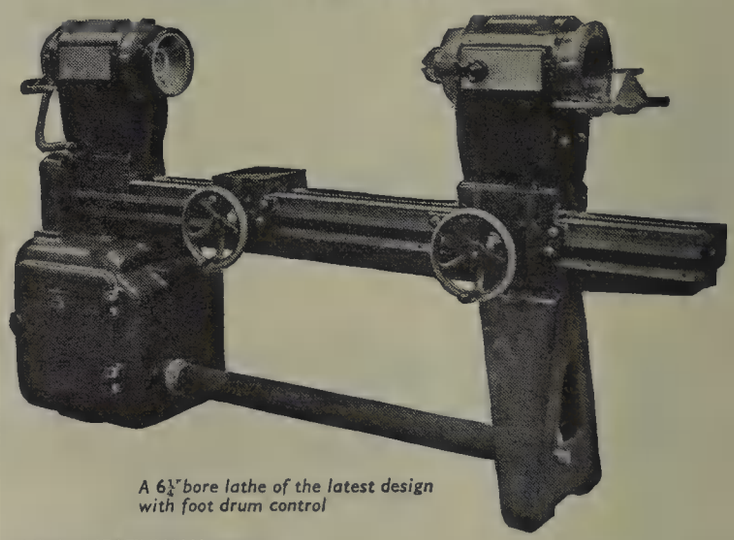

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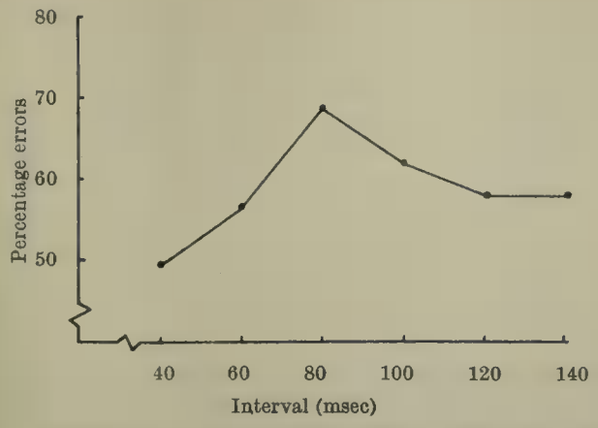

Fig. 2. Relationship between percentage errors and time-interval for all 6 subjects in the third experiment

taneously or with an interval of 50 msec than they do when the interval is 100 msec, both curves showing maximum errors at this time. Studying the error curves for each subject showed that 10 out of the 12 had a peak in their error scores at the 100 msec interval. The other 2 subjects were both in the descending group and both had a peak in their error curves at the 50 msec interval.

In an attempt to narrow the limits within which the peak difficulty occurred, a third experiment was undertaken. Using a Latin square design to balance practice and fatigue effects at the different time-intervals, 6 new subjects undertook 6 sessions, each of 2 practice sets and 4 test sets, each set consisting of 36 pairs of stimuli, arranged in a balanced design, as in the previous experiment. Each session was at one of the following 6 intervals: 40, 60, 80, 100, 120 and 140 msec.

The error curve for the group is given in Fig. 2. The results clearly confirm the findings of the previous experiment and indicate that the peak difficulties lie between 60 and 100 msec.

In this last experiment each subject undertook one session at each time-interval, the order of time-intervals being different for each subject. Practice effects between sessions were marked and the experiment provides no evidence on individual differences.

The analysis presented here takes no account of the numerosity of the stimuli or of order effects in relation to the relative numbers of neons in the first and second stimuli. Errors, as might be expected, were more frequent with the larger total numbers and were greater when the larger number was presented first, as contrasted with the situation when the second stimulus was the larger. These findings will be presented and discussed in detail later, but clearly the time-course of the error curve calls for brief comment.

In an earlier investigation on reaction time responses to successive stimuli separated by short intervals, it was observed that delays in the second response were not maximal at the shortest intervals[3]. This observation was critical to Welford's theory of a psychological refractory period, although Hick and Welford[4] suggested that the apparent exceptions to the theory might be due to subjects' grouping, or responding to both stimuli as a single unit, at the very short intervals. In the work recorded here it is obvious that, with almost no clues for a spatial separation, subjects must be grouping the stimuli when presented simultaneously. It is also clear that at the longest intervals the stimuli are treated as independent. The poor performance at the 50 and 100 msec intervals cannot be due to a grouping strategy. For the ascending group it represents a failure to maintain such a strategy as the interval increases. For the descending group it may be seen as a failure, as the intervals decrease to attain a grouping strategy which is achieved at the shortest intervals.

As will be reported elsewhere, each stimulus impairs the perception of the other and this can be related to the phenomena of metacontrast and of apparent movement. Subjects frequently reported apparent movement effects at the short time-intervals. The perception of two discrete stimuli as a single stimulus in motion would clearly impair the perception of numerosity. Unfortunately, the literature on the time relationships of both metacontrast and apparent movement is, in both instances, complex. However Alpern[5], who among others has reviewed the work on metacontrast, himself demonstrated a maximum masking effect when the interval between the stimuli was in the region of 100 msec.

While it is tempting to interpret the failures in the perception of numerosity, in terms of the apparently more basic processes of metacontrast and apparent movement, it is perhaps more useful to relate all these to the characteristics of central interpretative mechanisms. It is hoped to develop this approach in relation to a more detailed description of the present findings.

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CONTROL OF THE COMPETENT STATE IN *Pneumococcus* BY A HORMONE-LIKE CELL PRODUCT: AN EXAMPLE FOR A NEW TYPE OF REGULATORY MECHANISM IN BACTERIA

By Dr. ALEXANDER TOMASZ
The Rockefeller Institute, New York

COMPETENCE or 'transformability'—the ability of some bacterial cells to take up biologically active DNA molecules and undergo genetic transformation—is a genetically determined property of some *Pneumococcus* strains. It has been known for some time that in pneumococcal cultures passing through a typical growth cycle this genetic potency gains phenotypic expression in most of the cells only during a relatively short 'competent' phase of growth[1]. During this short phase, the cell population becomes highly uniform with respect to competence: the majority of the cells can be transformed by multiply-marked DNA, and there is evidence that all the individual cells present express the competence property.

The time course of this expression during growth shows some unique features: once an induction period is passed in which competence is low or undetectable, there is an abrupt and fairly 'synchronous' development of transformability at a rate very much faster than the growth rate of the culture. Under these conditions, the maximal competence is usually followed by a rapid decay of this property. A typical time course of such an expression process is illustrated in Fig. 1.

The cause of the delayed and abrupt appearance of competent cells in pneumococcal cultures has been a puzzle ever since the discovery of the existence of a 'competent phase' during which these bacteria are capable

of undergoing genetic transformation[2]. Since in some complex growth media the competent phase of pneumococci seemed to develop close to the onset of the stationary phase of growth, it was suggested that transformable cells are physiologically abnormal organisms (for example, cells deficient in ability to grow) which accumulate in the culture as the growth medium becomes exhausted in some nutrients[3,4].

However, more recent investigations revealed no support to this interpretation. Instead, it was proposed that the rapid accumulation of competent cells should be regarded as a shift to a distinct physiological state[5]. This point of view has already been expressed by a number of authors[2,6], but our experiments yielded new evidence in support. (1) It was found that pneumococcal cultures grown in chemically defined media develop the competent phase in a critical cell concentration range, but independent of the 'closeness' of the stationary phase or the growth rate of the culture[7,8]. (2) Competent pneumococci seem to be actively growing cells as indicated by their sensitivity to penicillin[9] and by the fact that the competence of a culture can be stabilized in a chemostat under conditions where slow-growing cells would be rapidly diluted out[7]. (3) Finally, the detection of small quantities of biologically active DNA in sterile filtrates of growing pneumococcal cultures near their peak of competence suggests that this property has a biological 'value' for *Pneumococcus* in that it may equip these bacteria with a mechanism for gene exchange[10]. In this unique recombination mechanism, gene transfer depends on the random collision of DNA molecules and cells. Since the rate of this process is a function of the cell concentration and the DNA concentration as well, the synchronous appearance of competence in the majority of the cell population makes the chances for genetic transformation optimum.

If one accepts this interpretation, then the development of competence may be regarded as the phenotypic expression of an important pneumococcal character which at low cell concentrations is 'repressed' in most members of the cell population.

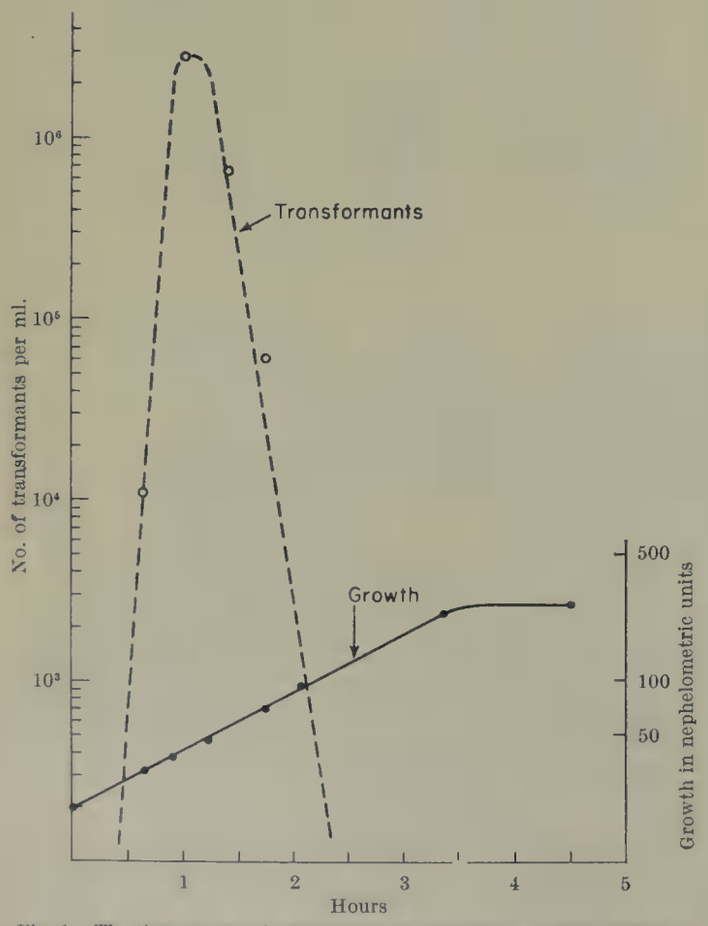

Fig. 1. The time course of development of competence during growth of a *Pneumococcus* culture

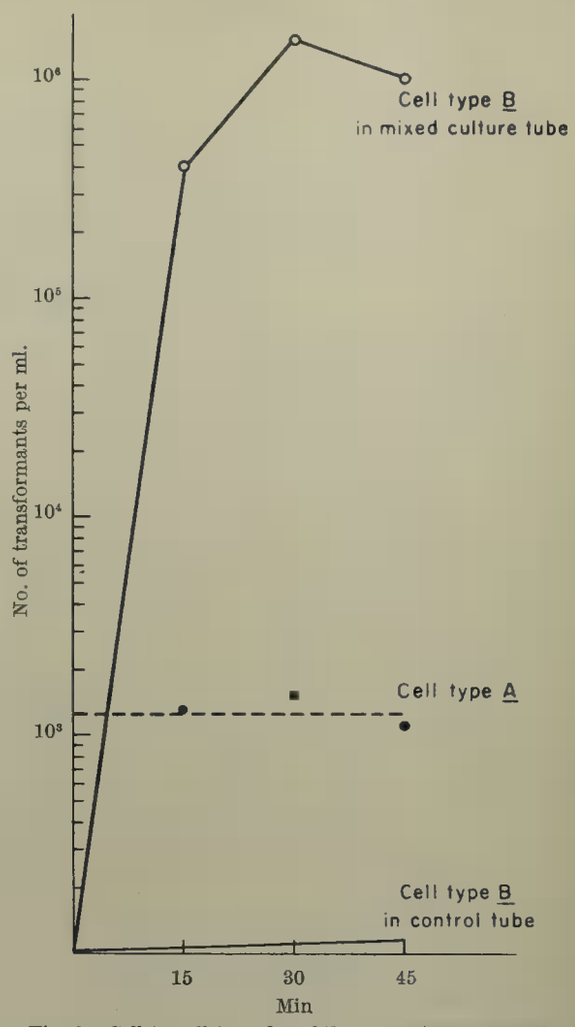

Fig. 2. Cell-to-cell transfer of the competence property

In the past three years, the mechanism of this delayed and synchronous expression was the subject of systematic investigations undertaken in our laboratory. It became apparent that the abrupt expression of competence does not depend on a general phasing of the life-cycles of the individual cells, since there is no detectable sign of massive division-synchrony in cultures during the development of competence.

It seemed likely, therefore, that the uniform and synchronous expression of this pneumococcal character is ultimately controlled by some environmental or endogenous factor able to affect randomly dividing cells.

Investigations of the effect of culture conditions on the time course of development of competence have clearly shown that alteration of the medium composition by the bacterial growth (for example, 'detoxification' or selective removal of some medium components) was not responsible for the synchronous 'expression' of this property[8]. Furthermore, as has already been mentioned, cultures of pneumococci growing in synthetic media in which growth was regulated by varying levels of enrichment were found to develop competence at about the same cell concentrations regardless of the phase of growth or rate of growth of culture[7,8].

Recent attempts to identify the mechanism controlling the expression of competence led to the discovery of a unique regulatory mechanism. It was found that the expression occurs through a co-operative process in which cells in the competent state can transfer this property to incompetent bacteria in a rapid process mediated by a specific macromolecular product of the competent cells[5]. The essential features of this observation are reproduced in the experiment illustrated in Fig. 2. Washed suspensions of two pneumococcal cell types which were distinguishable by their different antibiotic resistance (resistance against sulphanilamide in cell-type *A* and resistance against micrococcin in cell-type *B*) were mixed in a test-

tube in fresh medium. Cells of type *A* were highly competent and were introduced into the test-tube at a concentration of 1×10^6 cells per ml.; cells of type *B* were incompetent and were present at a concentration of 1×10^7 cells per ml. The same concentration of the incompetent cells was also introduced separately into a (control) test-tube without cell-type *A*. Both tubes were incubated at 30° C, a temperature at which the spontaneous development of competence is inhibited[5], and the transformability of the two cell types was determined by exposing aliquots to a DNA carrying the streptomycin resistance marker. The transformants were selectively scored in two different antibiotic-containing media: in medium *A* (containing sulphanilamide and streptomycin) to count transformed *A* cells and in medium *B* (containing micrococcin and streptomycin) to count transformed *B* cells.

Fig. 2 shows that whereas cell-type *B* incubated alone in the control tube remained incompetent, the same bacteria in the mixed culture tube rapidly developed a high level of competence as a result of some interaction with the competent cells of type *A*. There was no significant change in the numbers of cells present during the short span of the experiment. It was calculated that about 80–90 per cent of all the type *B* cells have become competent by the 'activating' effect of cell-type *A*, in spite of the fact that these latter made up only about 10 per cent of the total population. The competence of the type *A* culture did not change during this process.

Next the mixed culture experiment was repeated in a modified form: the cultures of both cell types were introduced into the separate compartments of a U-shaped glass tube which was divided in two compartments by a 'Millipore' membrane. During incubation at 30° C, the filtrates of the two cultures were mixed by continuous pumping of the medium through the 'Millipore' membrane. Under these conditions, physical contact between the competent and incompetent bacteria was excluded. Table 1 shows that the cell-to-cell transfer of competence did not necessarily require physical contact of the organism but could occur through the mediation of some filterable cell product. The relatively low efficiency of competence induction was attributed to the low concentration of cell-free 'activator' substance and its extreme lability[5].

Table 1

Cell type	No. of transformants per ml. after incubation for 30 min	60 min
B (incompetent) alone	0	0
B in mixed culture with *A* (competent)	$5{\cdot}7 \times 10^4$	$5{\cdot}7 \times 10^4$
B in U-tube with *A*	300	$1{\cdot}3 \times 10^4$

Cell-to-cell transfer of the competence property by way of a soluble cell product.

The activator substance, responsible for the cell-to-cell transfer of competence, was afterwards isolated from competent pneumococci. Purified preparations of this heat-labile material could convert incompetent bacteria to competence in a fast 'reaction', the rate depending on the concentrations of both the activator and the cells[5].

The *p*H optimum of this process was about 7·7, and the biological activity of activator preparations could be greatly enhanced in the presence of 0·05–0·07 M mercaptoethanol. Low concentrations of various proteolytic enzymes (0·1 μg/ml.), such as trypsin, chymotrypsin and subtilisin, inhibited the activation of incompetent cells by activator preparations, whereas these enzymes had no effect on the transformation of already competent cells by DNA. This observation suggested that the activator is a protein. Fractional elution of the activator substance from the cell debris yielded preparations with high specific activity: extracts with less than 0·2 μg protein/ml. could convert 10^6 cells to the competent state at 37° C in 10 min. The activator substance appears to be of fairly high molecular weight, since it is not retained during chromatography on 'Sephadex *G*25' columns. A small retention can be achieved on 'Sephadex *G*75' (Fig. 3).

The activator seems to be specific for *Pneumococcus*, since *Bacillus subtilis* and *Haemophilus influenzae* cultures in their incompetent phase of growth could not be converted to competence by pneumococcal activator. During the activation process the surface of the pneumococcal cells seems to undergo significant changes: a new antigenic determinant appears[11] and the bacteria develop a capacity to bind DNA and become transformed by it[12]. Subsequent investigations have shown that the rapid disappearance of competence which usually follows closely the brief maximal competence of the culture can be correlated with another cell-produced factor, called 'inhibitor'. After pneumococcal cultures reach a certain density their filtrates rapidly become enriched in this inhibitor which can block the action of the activator and prevent further spread of competence[5]. Thus, it appears that the transient and synchronous expression of the competence character during growth is controlled at the level of the pneumococcal population by extracellular factors manufactured by the cells themselves.

The attainment of a transient physiological state in a population by such an 'induction' process seems to be a novel phenomenon among bacteria, and it raises a number of interesting questions concerning the nature of the cell interactions involved in such a process. (It is important to distinguish between the cellular 'communication' involved in the propagation of competence and the cell interactions which occur during the cell-to-cell transfer of episomal or chromosomal material. These latter interactions serve the 'dispersal' of genetic material in a population of bacteria while in the case of competence a physiological state is propagated.)

In the traditional view bacterial populations are characteristically lacking mechanisms for specific intercellular communication. This is contrasted with cell populations of higher organisms in which hormonal mechanisms are known to induce and reinforce the expression of many physiological properties and control differentiation.

The control of competent state in pneumococci forces one to revise this view. Since the activator—a cell-produced chemical—seems to impose a high degree of physiological homogeneity in a pneumococcal population with respect to competence, one is forced to conclude that in this case bacterial population can behave as a biological unit with considerable, although only temporary, coordination among its members. In a sense, this situation then resembles hormonal control—an example for a primitive, temporary 'differentiation' in bacterial populations.

One wonders whether this kind of control may not be operative in some other microbial phenomena also. It is

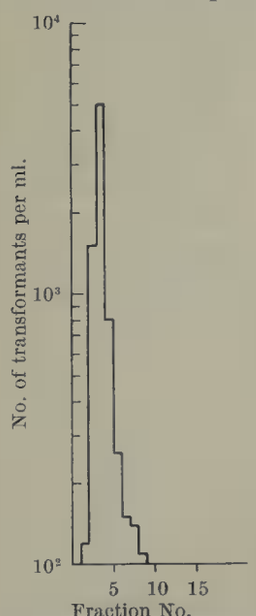

Fig. 3. Chromatography of pneumococcal activator on 'Sephadex *G*75'

well known that bacterial populations are able to undergo drastic changes in the physiological properties of the majority of the cells. Numerous examples for such massive non-genetic variability or 'modulations'[13] are known: the list includes the variation in cellular components such as amounts of capsular material, intracellular lipid, carbohydrate and polyphosphate inclusions, qualitative and quantitative changes in the surface antigens and fimbriae (for review see ref. 14), cyst formation[15], cyclic morphogenetic changes in cell shape[16], sporulation[17], changes in virulence[18], changes in the biosynthetic capacity as it occurs in the production of tyrocidine[19], bacitracin[20], edein[21], and other bacterial polypeptide antibiotics.

In the absence of known endogenous causes, such as genetic variation or biological clock mechanisms, such gross physiological changes in a whole bacterial population are usually considered as the results of interactions between cells and their environment (using the term 'environment' in its broadest sense), even though in the majority of the cases the environmental signal invoking them is not known and the physiological significance of the change is usually little understood.

For the interaction between environment and cell population, two general and contrasting types of mechanisms can be considered. In the first type, each individual in the population would receive and recognize the environmental stimulus and respond to it in a continuous interaction with the environment and without any interaction with other individuals in the population. The homogeneity of the population with respect to the particular physiological state would then be the result of the basic uniformity of individuals as well as the uniformity of the environmental change. The derepression of metabolic pathways by the depletion of an end-product is at present considered as having such a mechanism.

In the second type of mechanism, a similar physiological response would originate in a radically different way. The environmental change would be first recognized by a small number of cells only; these cells would respond by 'converting' this environmental signal to a second and specific extracellular (chemical) signal, for example, the activator substance in the case of competence, which in turn would interact with the rest of the individuals of the population and convert them to the appropriate physiological state. In this 'hormone-like' mechanism, the physiological change in the majority of the cells does not occur by virtue of a continuous interaction with the environment but rather is called forth by a specific cell product. The homogeneity of the population with respect to the physiological character is determined here primarily by the specificity and effectiveness of the activator. (The terms 'hormone' and 'hormone-like' are used here in their broadest sense to indicate specific cellular products which have the biological function of co-ordinating individual cells of a cell population with respect to a physiological property. While this formulation includes the essence of the hormonal function, it clearly differs from the traditional definition of 'hormone' which restricts the use of this term to internal secretions of multicellular organisms. The need for a less stringently defined term for hormone-like functions was discussed in detail by J. Huxley, who proposed that such substances could, perhaps, be generally called 'activators'. 'Activator' should include all "chemical substances produced by organisms which exert specific functions in regard to correlation or differentiation"[22].)

The 'hormone-like' role of activator in the cell-to-cell propagation of competence seems well established. Practically nothing is known, however, about the nature of the stimulus invoking the production of this substance. It was found that in a number of experimental systems, the prolongation of the induction period preceding the appearance of competent cells is caused by the apparent failure in the production of endogenous activator[8].

Control by an extracellular activator is not restricted to the transformation system of *Pneumococcus*. A similar induction system has been discovered independently by Pakula and Walczak[23] among some transformable strains of *Streptococcus*, and we found evidence for the existence of an activator of the pneumococcal type in a streptococcus strain[12] which is closely related to *Pneumococcus* (*Streptococcus viridans D* used in the work of Bracco *et al.*[24]). More recently it was reported that the competence of a transformable *Bacillus cereus* strain also is regulated by an activator substance[25]. Possibly, other transformation systems will be found to have similar cellular regulatory mechanisms.

At present, however, there seem to be only a few other well-established examples of such activator-mediated 'cell communications' among micro-organisms, and the control of competence in *Pneumococcus* and *Streptococcus* seem to be the first examples among bacteria in which such a mechanism can be clearly outlined.

Perhaps the most clearly understood other case is the chemotactic movement of some myxomycetes during their morphogenetic aggregation[26,27]. A somewhat similar effect has been described in the induction of sporulation of vegetatively growing *Bacillus subtilis* by extracts prepared from sporulating cells[28]. It may be worth while, however, to suggest the possibility that activators may also participate in the regulation of some bacterial 'phenotypic variations', a number of which have already been listed. Judging from the homogeneity of the competence property which the activator produces as well as from the extremely fast rate of the activation process, such activator mechanisms could greatly improve the uniformity and efficiency of cellular responses to some environmental change and in this way improve the adaptive potential of populations.

Another phenomenon which may perhaps be interpreted within the framework of such an activator mechanism is the loss of virulence of group-*A* streptococci during growth under reducing conditions. The value of this physiological change for the organism is not clear, but the mechanism of this effect may be taken as an example of how a rapid change in a property of a bacterial population may be coupled to an environmental change. Elliott has shown[29] that, in this case, the loss of virulence is associated with the loss of the antigenic *M*-protein from the cell surface as a result of the action of an extracellular protease. This enzyme is excreted by the cells in an inactive zymogen form and becomes activated by the reducing conditions produced in the anaerobic environment.

Clearly, an enzyme could be an ideal activator for the efficient regulation of a property in a cell population, and it may be interesting to consider the role of some bacterial exoenzymes as instruments in the regulation of some as yet unrecognized physiological states. If this were valid, then it is likely that the targets of such exoenzymes or other specific extracellular cell products would be primarily surface components of the bacteria. Interactions with bacterial surface structures can have extremely specific and far-reaching intracellular consequences, as is known from investigations of the mechanism of action of colicins[30] and adsorbed bacteriophage ghosts[31].

Interactions between groups of cells through specific cell-products is, of course, quite common among higher organisms on all levels of cell associations and even in ecological relationships[32]. There are numerous examples of hormonal control in morphogenesis and differentiation, as well as in the 'integration of individuals of a population' through specific extracellular chemicals[33]. The results discussed in this article suggest that the capacity for such a population-level control may already exist among micro-organisms. In addition to the highly efficient enzymatic control mechanisms (such as end-product control or repression), which operate in 'individual' cells of bacteria, 'populations' of these organisms may exhibit supracellular regulatory mechanisms also, which can efficiently control a physiological property in a large number of cells. At present, it is not known whether the pneumococcal

activator participates in the regulation of gene activity or operates entirely on the physiological level.

The fact that the transformable state in *Pneumococcus* is regulated by a specific cell product may be interpreted as emphasizing the evolutionary importance of transformation for *Pneumococcus*. This investigation was aided by a grant from the National Science Foundation (*GB*—2083) to Dr. R. D. Hotchkiss.

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RATE OF CHROMOSOME DUPLICATION AT THE END OF THE DEOXYRIBONUCLEIC ACID SYNTHETIC PERIOD IN HUMAN BLOOD CELLS

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IN an earlier publication we considered the pattern of chromosome duplication at the end of the DNA synthesis period (*S* period) in human blood cells[1]. One of the notable features was a difference in the rate of DNA synthesis between certain chromosomes, particularly so in one of the *X*-chromosomes of females, the 'hot' *X*-chromosome. Because of the intensive labelling of this chromosome at a time when most other chromosomes were unlabelled or only weakly labelled, the 'hot' *X* has been considered as being delayed in its duplication[1-4]. This article analyses in more detail the labelling behaviour of the autosomes as compared with the 'hot' *X*-chromosome at the end of the *S* period.

Cultures of female peripheral leucocytes were prepared by the usual method[5] with the difference that red cell agglutination was carried out by letting the sample stand (1 h) at room temperature and using phytohaemagglutinin *M* (Difco) only as a mitotic stimulant.

Labelling was carried out at minus 3 h with 0·2 μc./ml. medium of tritiated thymidine (Radiochemical Centre, Amersham, spec. act. 12·3 mc./μM). 'Colcemid' (Ciba, 0·04 ml./ml. medium) was added 1·5 h before termination of the cultures. Slides were prepared for autoradiography with Kodak '*AR* 10' stripping film and exposed for 8 days.

A random sample of 224 cells in metaphase was selected for analysis. To ensure a proper random sample, cells in metaphase were rejected only for one or more of the following reasons: (*a*) an incomplete set of chromosomes; (*b*) an excessive overlap of any chromosomes that prevented them from being individually identified; (*c*) the cell crossed by a prominent 'background streak' of grains. Except for the criterion (*c*) the autoradiographic grains were ignored during selection.

After the selection of the cells had been made the autoradiographic grains over the whole sample were counted by two observers. For each cell the grains were put into three mutually exclusive classes: (*a*) *background grains*, grains which were not over any chromosome but within the boundaries of the metaphase plate; (*b*) *'hot' X grains*, grains over the most heavily labelled chromosome in Group III (Denver classification); (*c*) *autosome grains*, all the other grains, that is, grains over all chromosomes except the 'hottest' in Group III.

A grain was taken to be over a chromosome if it was within a distance of one chromatid width. For the sake of brevity 'autosomes' will include the 'cold' *X* chromosome.

The results are shown in Table 1 and Figs. 1–3. For the purpose of analysis two distributions are shown: (*a*) cumulative distribution of the total grains over the cell, excluding the 'hot' *X*, that is, the sum of classes (*a*) and (*c*) (Table 1 and Figs. 1 and 2); (*b*) cumulative distribution of the grains over the 'hottest' chromosomes in Group III, that is, class (*b*) (Figs. 1 and 2).

(1) *Unlabelled cells.* From Table 1 we can infer that between 160 and 165 cells were unlabelled, and that the grains observed over these cells arise from a random distribution of background. Table 1 shows a distinct change of pattern for cells with 12 or more grains. These cells show labelling over the 'hot' *X*, which is markedly higher than for cells with less than 12 grains, indicating positive labelling. The background is about 2 grains per cell,

Table 1. Grain Count Data of the 224 Cells

Grains over autosomes + background	Cumulative No. of cells	Mean grains per cell		
		Autosomes + background	Background	'Hot' *X*
0	3	0	[illegible]	0·3
1	14	1	0·6	0·7
2	41	2	1·1	0·9
3	65	3	1·5	0·9
4	94	4	2·2	0·9
5	113	5	2·2	1·3
6	129	6	2·9	1·2
7	145	7	3·2	1·1
8	155	8	4·2	0·9
[illegible]	161	9	5·5	1·8
10	164	10	5·3	2·6
11	167	11	5·0	1·3
12–13	171	12·7	1·75	9·3
14	176	14	1·0	14·5
15–20	184	17·1	2·0	8·8
21–100	205	50·9	3·2	22·6
>100	224	242·1	5·1	40·3
0–9	161	4·28	2·12	1·01

agreeing with a random background of 2·12, the mean for cells with 9 grains or less. That the majority of the 167 cells with 11 grains or less are unlabelled is indicated by the fact that the 'hottest' in Group III has only on average about one grain, and also that about half the grains over the cells are background. However, a few of these 167 cells will nevertheless have some real label as four of them had a Group III chromosome with 5 or more grains.

Taking therefore the 161 cells with 9 grains or less as representative of the background population, a detailed analysis was made to check that these grains were randomly distributed over the cell and over the chromosomes. The mean grains per cell for this population is 4·28 (Table 1) with a mean background of 2·12, giving therefore 2·16 over the autosomes. This latter, together with 1·01 grains per cell over the 'hottest' in Group III, makes a total of 3·17 grains per cell over all the chromosomes. Since Group III contains 37·8 per cent of the chromosome material, a random distribution of grains would lead to an expected mean over Group III of 1·20. Since even

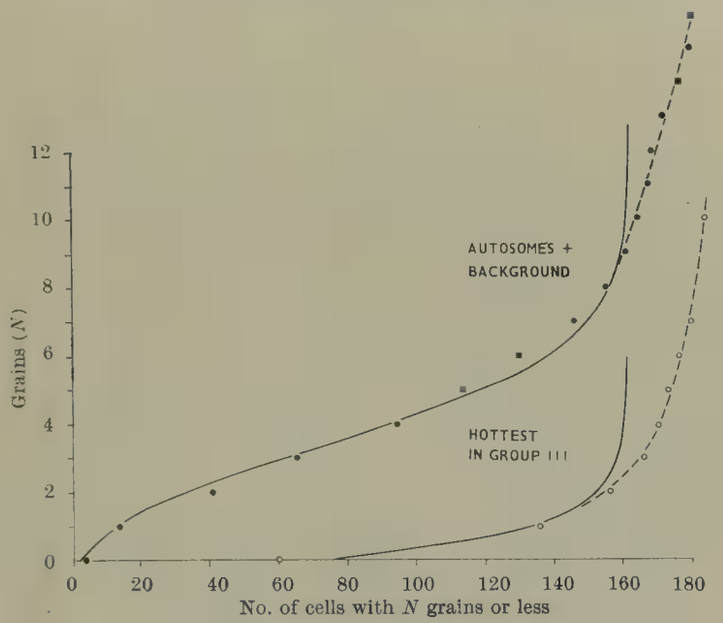

Fig. 1. Cumulative distributions of grains over the unlabelled cells. Solid lines show the Poisson distributions of 161 cells with mean grains of 4·28 and 0·72

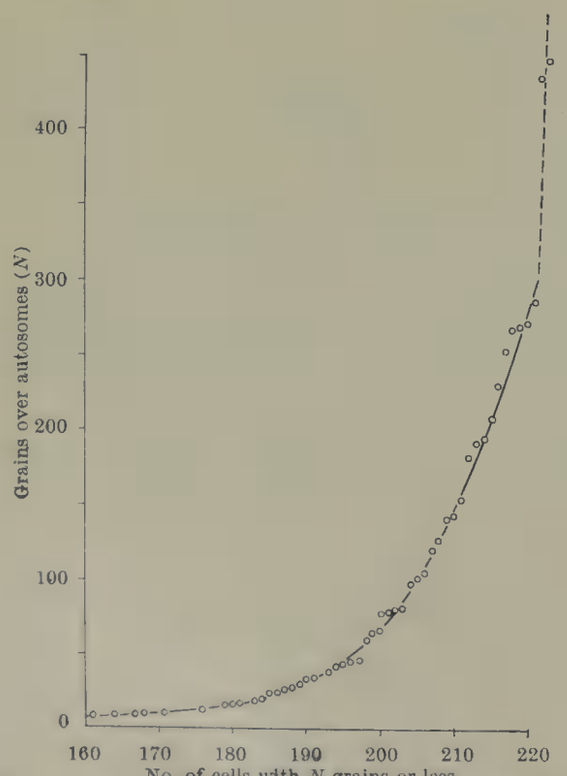

Fig. 2. Continuation of the cumulative distribution of the grains over the autosomes (and background) to include the labelled cells

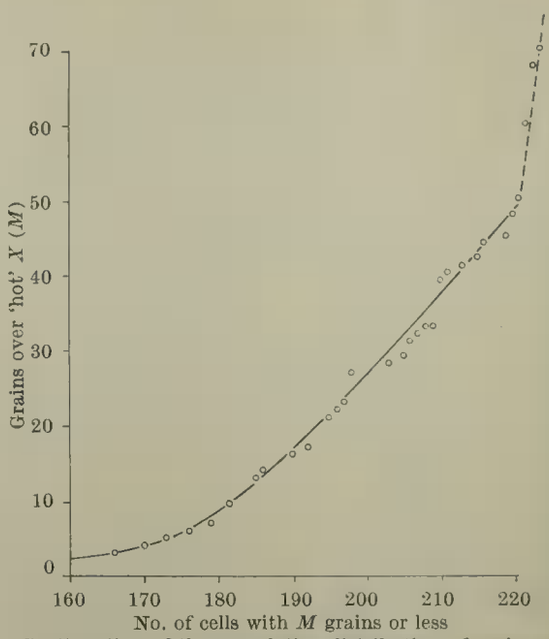

Fig. 3. Continuation of the cumulative distribution of grains over the 'hot' X to include the labelled cells

with this low density, occasionally more than one chromosome in Group III will be labelled, the observed value of 1·01 over the 'hottest' is consistent with an expected value of 1·20 over the whole group. Furthermore, the distribution of grains among these cells agrees with a random distribution as is shown in Fig. 1, where the solid lines are the calculated Poisson distributions for a population of 161 unlabelled cells with a total mean count of 4·28 grains, and for a population of 161 Group III chromosomes with a mean grain count of 0·72. This latter is the mean of the smallest 161 counts over the 'hottest' chromosome in Group III; this distribution of cells being not necessarily identical with the tail of the autosome and background distribution.

(2) *Labelled cells.* From these arguments we conclude that the cumulative distributions shown in Figs. 2 and 3 are those for the labelled cells. These distributions can be interpreted by considering the consequences of the 'Colcemid' block. Without 'Colcemid', only cells which were 180 min from metaphase when the label was added will give metaphase plates. However, with 'Colcemid' added 90 min before fixation, all cells which were between 90 and 180 min before metaphase when the label was added will give metaphase plates. The proportion of these plates which show label will depend on where in the interval, 90–180 min, the DNA synthesis ends.

Since we have observed a cohort of 224 cells by the 90 min 'Colcemid' block, and since we can also assume these to be distributed uniformly in time, then these cells were coming into metaphase at 224/90 = 2·49 cells per min. Thus, a time-scale can be put on the cumulative distributions of Figs. 2 and 3, the abscissa now becoming a linear time-scale going backwards into the S period. The ordinate will be proportional to the total DNA synthesis still to be completed, since the tritium label is continuously available during this incubation period, and when once incorporated in the chromosome is permanent, at least until the next cell division.

The tangents to the cumulative distribution curves, therefore, give a measure of the rate of DNA synthesis at the corresponding times. In this way the rate of DNA synthesis at the later part of the S period can be calculated for the 'hot' X and for the autosomes, and the results are shown in Fig. 4. Since the cumulative distributions give the total grains over the 'hot' X and over all the autosomes, the rate of increase of these grains has been divided by the length of the X chromosome, or by the total length of the autosomes, to get a specific rate of DNA synthesis, that is, a mean rate of synthesis per unit length of chromosome.

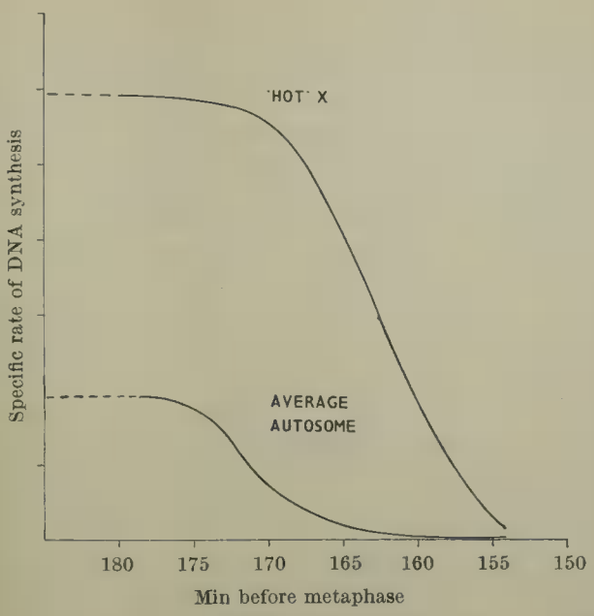

Fig. 4. The specific rate of DNA synthesis at the end of the *S* period

Individual cells will show a variation about the mean values of Fig. 4. One effect of this variation is to give the sharp upward tilt to the ends of curves of Figs. 2 and 3. This is due to a few cells being farther back in time in the *S* period than the average cell at 180 min before metaphase. From these few cells we can make a rough estimate of the average value of this time, by taking the time between the extrapolated curve and the actual curve at a grain count equal to the mean for these cells. This time corresponds to about 5–6 min for both the 'hot' *X* and for the autosomes.

Since the two cumulative distributions for the 'hot' *X* and the autosomes are independent of each other, we can examine where each individual cell occurs in the two distributions. There is a good degree of correlation and this can be expressed as the mean ranking difference of a cell in the two distributions and was calculated to be 10·8 cells. Since the cohort is progressing at 2·49 cells/min, this corresponds to 10·8/2·49 = 4·4 min internal timing variation between the state of the 'hot' *X* and of the autosome DNA synthesis within a single cell.

The results obtained in these experiments with continuous labelling by tritiated thymidine indicate that the rate of DNA synthesis is constant towards the end of the *S* period and then falls off sharply during the last 20 min. Our observations were limited to the last 25–30 min, therefore we cannot say how far this constant rate extends back into the *S* period.

Several publications have commented on the 'lateness' in labelling of the 'hot' *X* chromosome[1–4,6]. This was shown by an increasing proportion of the grains located over the 'hot' *X* as the total grain count over the cell decreases[1,2,6].

The results recorded here offer a quantitative explanation of this phenomenon. The specific rate of DNA synthesis is about three times higher in the 'hot' *X* than in the average autosome (during the last constant part of the rates). This constant rate is maintained somewhat longer in the 'hot' *X* than in the average autosomes (by about 5 min). The rate in the 'hot' *X* then falls sharply so that both the 'hot' *X* and the average autosomes finally finish at about the same time, in this culture about 155 min before metaphase.

Although the two rates of synthesis end together, the ratio of the areas under the two curves in Fig. 4 is about 6, corresponding to the specific grain density of 6 for the 'hot' *X* reported earlier[1,6].

In order to single out the 'hot' *X*, all other chromosomes have been grouped together under the term 'average autosome'. The known differences in grain density between the various autosomes and parts of autosomes indicate that any one segment of any chromosome may have its own 'end of *S*' pattern. Some of these may not differ greatly from the 'hot' *X*, others may be greatly displaced early in time. For these reasons the curve for the average autosome might well be expected to have a more gradual ending than that of the more homogeneous 'hot' *X*.

It would require a very considerable amount of grain counting to provide data for any one autosome, let alone for parts of autosomes; but with suitably longer exposure of the autoradiographs it is possible to determine similar patterns at least for some of the autosomes.

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SHADE TOLERANCE IN FLOWERING PLANTS

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SHADE tolerance and shade avoidance. A distinction has been drawn[1,2] between species with seedlings which are able to penetrate rapidly through grassland shade (shade-avoiding plants) and species which are capable of surviving long periods in the dim light of the forest floor (shade-tolerant plants). Certain characteristics which facilitate rapid emergence from grassland shade have been recognized[2]. In this article, experimental results suggesting a physiological basis for shade tolerance are summarized.

Fungal attack and shade fatalities. Failure of seedlings in shade is almost invariably associated with fungal attack[2–7] and often the effect of shading is to increase infection[2,8–11]. Experimental evidence with tree seedlings[2] suggests that in comparison with tolerant species, shade-intolerant plants are more susceptible to infection both above and below the compensation point.

Predisposition to fungal attack. Correlations have been established between decreasing sugar content and increased susceptibility in shaded foliage[11–13] and a causal role in resistance to infection has been ascribed to sugar content[14]. Consistent with this theory is a recent observation that the longevity of tree seedlings in deep shade is closely related to the quantity of material stored in the seed (Table 1).

The carbohydrate economy of the shade-tolerant seedling is therefore of interest for two reasons. If high levels of sugars are maintained in shade, continued growth may be possible; in addition we may suspect that fungal attack will be resisted.

Morphogenetic adaptation to shade. Morphogenetic changes induced by shading include increased leaf area, reduced leaf thickness and laminae inclined at right-angles to the light source[2,15–17]. These changes appear

Table 1. Number of Fatalities recorded in Seedlings of Eight North American Tree Species grown in Deep Shade

	Seed reserve (mg dry wt.)	No. of fatalities
Betula populifolia	0·1	4·6
Betula lenta	0·7	5·4
Rhus glabra	1·5	4·8
Acer rubrum	7·3	3·2
Liriodendrom tulipifera	8·5	3·4
Pinus strobus	14·8	3·6
Gleditzia triacanthos	40·3	1·8
Quercus rubra	1,969·2	1·4

Each value tabulated is the mean number of fatalities per container over a period of twelve weeks. Initially, a single seedling was sown into each of five containers. Casualties were replaced by freshly germinated seedlings throughout the rest of the experimental period.

to be advantageous in that they increase the amount of energy intercepted by the shoot. They are not confined to shade-tolerant species[18], however, although in some investigations more effective responses to shading have been observed in shade-tolerant plants[17].

Metabolic adaptation to shade. Many shade-tolerant genera, for example, *Pachysandra* and *Hedera*, have thick leaves and leaf arrangements which permit considerable self-shading. Clearly this indicates that the carbohydrate solvency of shade plants may depend on conservation of energy rather than the efficiency of its capture.

Initial study of the metabolism of shade-tolerant plants has been carried out in Connecticut by measuring: (*a*) relative growth-rate of small plants of tolerant and intolerant species growing under full sunlight; (*b*) respiration in the dark in leaves of species differing widely in shade tolerance.

(*a*) Under the conditions of the experiment (Table 2) all the shade-tolerant species examined grew extremely slowly in comparison with the species intolerant of shade, crop plants and various weeds.

(*b*) In shade-tolerant species respiration rates of leaf disks in darkness were consistently lower than those measured in species from open habitats (Table 3).

From these data it would appear that shade plants have slow metabolic rates. It is not clear whether these are imposed biochemically or arise through reduced permeability of the leaf. As suggested by D. N. Moss, dark respiration may be inhibited by accumulated carbon dioxide within the leaf and photosynthesis by slow inward diffusion of carbon dioxide. As demonstrated by Heath[19], respiration may be inhibited by depletion of oxygen in leaves with tightly closed stomata. However, the alternative of control of photosynthetic and respiratory rates through common or linked slow biochemical processes is suggested by data, such as those of Thomas and Hill[20], which demonstrate a close correlation between rates of photosynthesis and respiration in species with high gas-exchange capacity.

Table 2. Relative Growth-rate (mg/g/h) of Shade-tolerant Species. Crop Plants and Species from Unshaded Habitats

Shade-tolerant species			
Tsuga canadensis	1·79	*Acer saccharum*	0·99
Castanea mollissima	1·41	*Pachysandra* spp.	0·92
Quercus rubra	1·66	*Deschampsia flexuosa*	0·57
Shade-intolerant trees			
Ailanthus altissima	3·32	*Rhus glabra*	2·97
Betula populifolia	9·24	*Fraxinus americana*	3·44
Betula alleghaniensis	5·02	*Paulownia tomentosa*	8·02
Betula lenta	4·75		
Crop plants			
Sunflower	6·53	Tomato	12·55
Cucumber	7·45	Sugar beet	11·64
Tobacco	31·39	Kentucky bluegrass	13·20
Dallis grass	16·71		
Arable weeds			
Ambrosia artemisifolia	15·46	*Portulaca oleracea*	12·43
Polygonum persicaria	10·19	*Rumex acetosella*	10·19

Each value tabulated is the mean of measurements taken with five replicates, over three consecutive five-day periods in June, 1964, New Haven, Conn. Plants grown on Arnon solution in sand culture in sunny weather.

Table 3. Dark Respiration (mg/g dry wt. Lamina/h) at 25° C in Leaf Disks of Shade-tolerant Species and a Variety of Plants from Unshaded Habitats

Shade-tolerant species			
Tsuga canadensis	2·19	*Acer saccharum*	1·80
Castanea mollissima	1·20	*Acer rubrum*	2·12
Quercus rubra	1·76	*Fugus grandifolia*	2·58
Taxus brevifolia	1·17	*Pachysandra* spp.	2·14
Hedera helix	2·36	*Viola* spp.	1·41
Shade-intolerant trees			
Liriodendron tulipifera	3·89	*Populus tremuloides*	4·17
Juglans nigra	2·70	*Betula lenta*	3·62
Paulownia tomentosa	5·13	*Rhus copallina*	6·62
Prunus serotina	4·80		
Crop plants		Arable weeds	
Tobacco	9·00	*Rumex acetosella*	7·26
Sunflower	3·51	*Atriplex* spp.	3·33
Bushbean	3·01	*Amaranthus retroflexus*	5·37

Respiration was measured as the loss in dry weight of disks from young fully expanded leaves floated on water for 12 h. Leaf samples were freshly collected from trees growing in unshaded habitats or from seedlings grown in sand culture in a greenhouse.

Recently high-temperature optima have been observed for photosynthesis in certain sun plants[21,22]. A high-temperature optimum for some fraction of respiration may be concomitant with this adaptation. Accordingly, in seedlings of *Paulownia tomentosa*, a shade-intolerant tree with an exceptionally high relative growth-rate in full sunlight (Table 2), leaf respiration was accelerated to a high rate over the temperature range 25°–35° C (Fig. 1). Values for a tolerant species of *Pachysandra* were comparatively low.

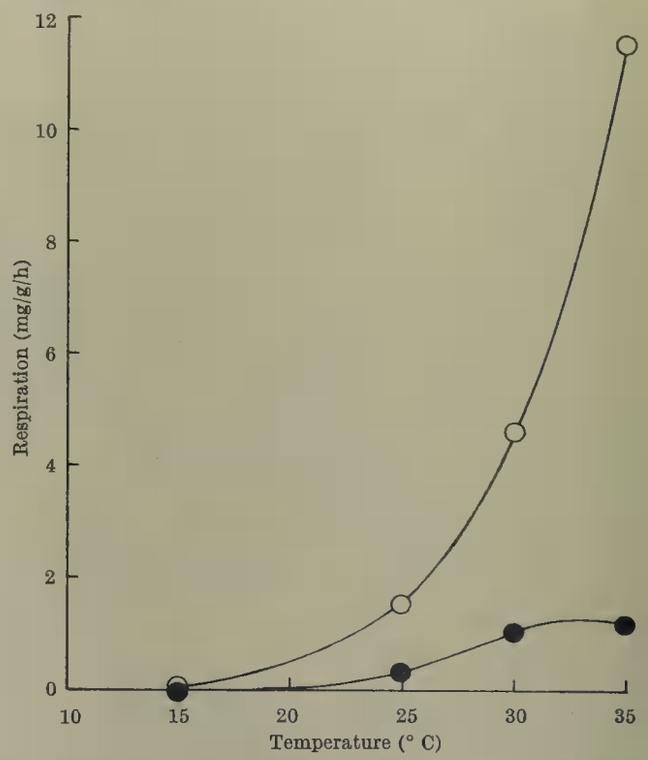

Fig. 1. The effect of temperature on respiration of leaf disks in darkness in *Pachysandra* (shade tolerant, ●) and *Paulownia* (intolerant, ○)

This investigation was carried out using plants grown in full sunlight. Provided the differences between tolerant and intolerant species, which have been observed in respiration rate, obtain in shade-grown plants, we may assume that depletion of carbohydrate below the compensation point will be more rapid in intolerant species. Furthermore, where intolerant species have a high Q_{10} for respiration, their compensation point will be most sensitive to changes in temperature. This was apparent in an experiment with *Paulownia*, in which a rise in temperature from 25° to 35° C caused a large depression in net photosynthesis at low light intensities (Fig. 2) and an elevation of light intensity compensation point from 300 to 1,200 ft. candles.

The contrast between tolerant and intolerant species in relative growth-rate and leaf respiration rate supports the suggestion of Went[23] that an important adaptation of shade plants has arisen through selection for slow respiration in shade with concomitant selection for slow photosynthetic rates and hence slow growth-rates in all environments. In accordance with this, photosynthetic rates of intolerant species exceed those measured in tolerant plants even when the comparison is made at low light intensities[17,24–26]. Apparently, these high rates of photosynthesis do not confer shade tolerance. This is

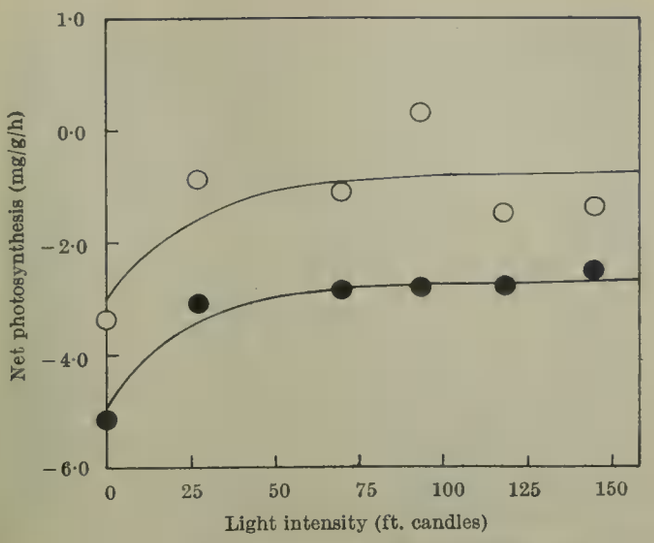

Fig. 2. Net photosynthetic rate in leaf disks of *Paulownia tomentosa* incubated in a range of low light intensities at 25° C (○) and 35° C (●)

probably because, in intolerant species, they are offset by high rates of respiration and by a rapid and continuous conversion of photosynthate into plant structure.

In a wider context, it has been proposed[27] that the plant characteristics which lead to the failure of a genotype or species in one environment may be an indirect consequence of adaptations necessary for survival in another. Hence high respiration rate, a liability in shaded habitats, is a feature of the high metabolic rate necessary in colonization of unshaded productive environments such as abandoned arable fields. The distinction between tolerant and intolerant plants may, therefore, arise through a physiological dilemma; adaptation for high productivity under full sunlight involves the assumption of characteristics limiting in shade and vice versa.

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THE CONCEPT OF THE 'CENTRON'

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THIS communication proposes a structural concept which has been found useful in accounting for the behaviour of cortical follicles during antigen stimulation of rat lymph node. Such changes can be explained by supposing that the follicular lymphoid cells are grouped in relation to a pre-existent spherical stromal structure. This stromal 'centron' is considered to be constant, though its contained lymphoid population is very variable.

The case for the existence of the centron depends on consideration of the following: (*a*) visible changes in follicular lymphoid cells under antigen stimulation; (*b*) visible changes in cortical macrophage processes under antigen stimulation; (*c*) flow patterns of afferent lymph-borne carbon and flagellar antigen in the lymphoid cortex.

Visible changes in lymphoid cortical follicles under antigen stimulation. Within the cortex of the rat lymph node can be found a series of follicles of variable cell population, depending on the age of the animal. These age changes in follicular cytology appear to be due to the accumulation of natural antigenic stimuli, as they can be reproduced by appropriate experimental lymph-borne antigen.

In young rats (70–100 g) the follicular system is represented by a series of surface mounds. Beneath each mound can be seen in the cortex a condensation of small lymphocytes, showing in many cases the beginning of a circular edge (Fig. 1). These small lymphocyte condensations are difficult to observe in ordinary paraffin sections, as they differ from the rest of the cortical lymphocytes only in the tightness of their packing. They are best seen in unfixed frozen sections cut from a node placed in a gutter in a block of liver or other supporting material.

In older rats, these follicles are more easily defined because they differentiate into a peripheral rim of closely adherent small lymphocytes, and a central area which contains numerous primitive blast cells with prominent

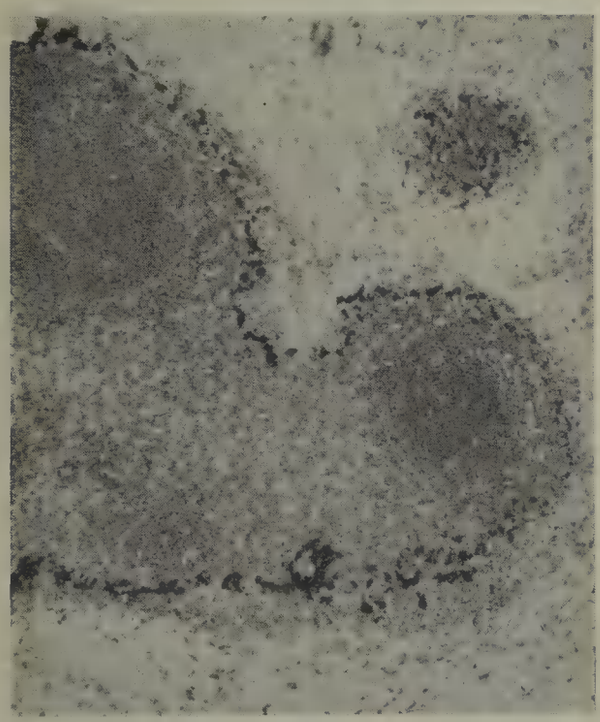

Fig. 1. Lymph node cortex from 100 g rat, with subcapsular sinus macrophages marked by afferent lymph-borne carbon. Note (*a*) the surface mounds, (*b*) the spherical masses of packed lymphocytes lying below. H. and E. 5μ frozen section. (× *c.* 112)

nucleoli. However, this blast cell concentration can be more controllably reproduced by experimental antigen stimulation of the 70–100-g rat (0·1 c.c. TAB vaccine, Commonwealth Serum Laboratories, into the right hind footpad twice a day for 5 days).

By the fifth day of this 'mass antigen' course, examination of the right para-aortic lymph node will show in the outer cortex a series of blast cell masses, forming incomplete circles, with the outer part of their circumference obscured by packed small lymphocytes extending to the subcapsular sinus (Fig. 2). If placed more deeply, or perhaps cut obliquely, these follicles are no longer obscured by lymphocytes, and appear as a full circle (Fig. 2). In this case there is a sharp contrast between the packed blast cells of the follicle and the loose lymphocytes of the cortex outside. The smooth and well-defined edge of the blast cell mass is very obvious (Fig. 2).

Observations of serial sections of many lymph nodes under mass antigen stimulation suggest that these blast cell circles correspond to sections of a sphere 200–250μ in diameter. Their constancy of shape and size, and the sharpness of their edge, are contrary to the idea that they are random aggregations. Instead, the appearances suggest that the blast cells fill pre-formed cortical stromal spheres, thus making them visible.

Visible changes in cortical macrophage processes under antigen stimulation. Pioneer researches of Bunting[1] and others at the turn of the century have recently been reinforced by modern electron microscopy[2,6] and the skeleton of the lymph node shown to be a protoplasmic pseudo-syncytium, made up of the interlacing membraneous processes of dendritic macrophages, with fibres running in extra-cytoplasmic tunnels and providing occasional reinforcement.

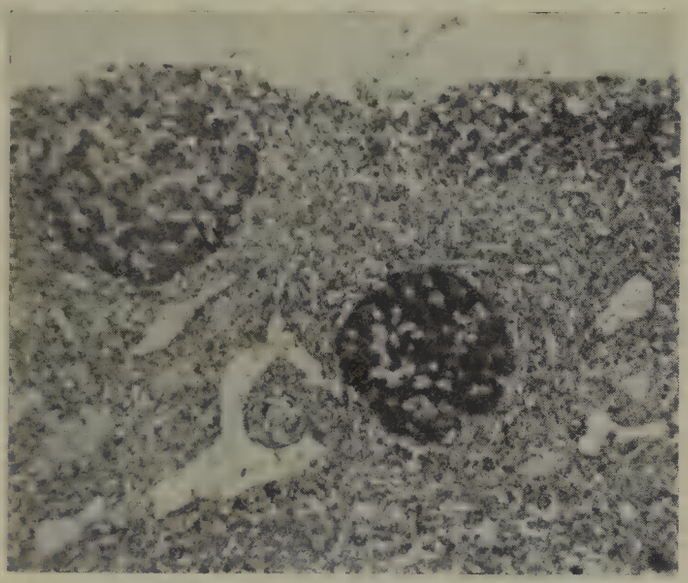

Fig. 2. Lymph node cortex after 5 days 'mass antigen' (see text), stained by a nucleic acid method (ref. 4) showing blast cells as dark masses. Two blast cell follicles are shown. On the left, one follicle is partly obscured on its outer aspect by packed lymphocytes. On the right, another follicle is seen with a fully circular shape. Note the sharpness and smoothness of the edge of this blast cell mass. Basic fuchsin-azure *B*. 3μ paraffin section. (×75)

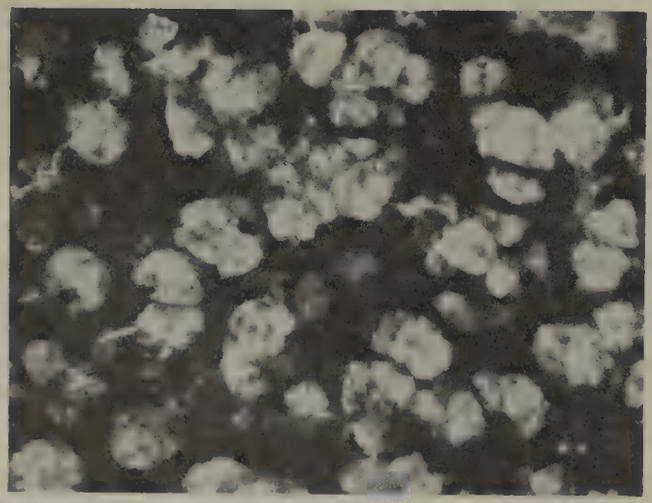

Fig. 3. Macrophages in the lymph node cortex, the processes of which have been outlined by adherent carbon, as described in the text. The ribbon-like processes broaden into macrophage bodies containing indistinctly visible nuclei. 10μ frozen section. (×900)

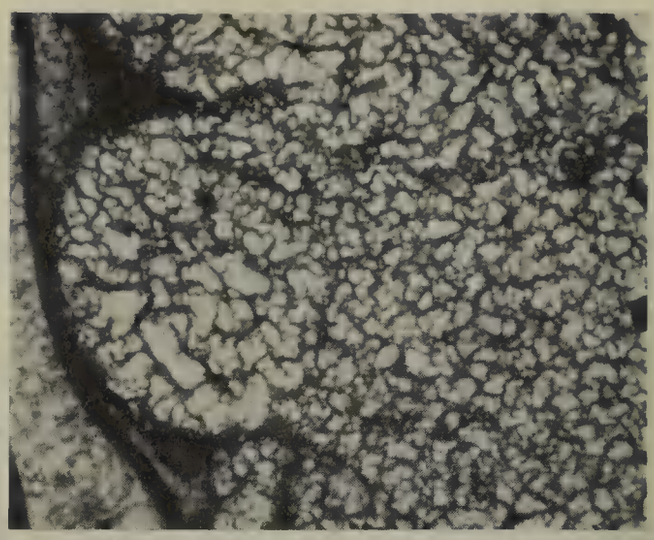

Fig. 4. Follicular mound (see left) from 100 g rat. Adherent carbon shows the general uniformity of the dendritic macrophage pattern, with no sign of follicular organization. 10μ frozen section. (×*c.* 170)

Fig. 5. Adherent carbon preparation of rat lymph node cortex one week after cessation of 'mass antigen'. Follicular areas of thickened dendritic macrophage processes are now easily seen. 10μ frozen section. (×75)

These stromal macrophage extensions can be outlined by the application of colloidal carbon. 10μ frozen unfixed sections of lymph node, dried on the slide at room temperature for 6–8 h, are flooded with 'Pelikan' indian ink (Gunther Wagner *C*11/1431*a*), for 10 sec and washed with a 4 per cent aqueous dilution of the same ink. This dilution is finally used as a mounting medium under a coverslip.

The carbon adheres selectively to the bodies and processes of macrophages, leaving lymphoid cells almost completely unmarked (Fig. 3). Observation has to be speedy, as the carbon particles dislocate from the macrophages after 15–20 min. Washing the preparation with water, as in any attempt to counterstain, results in the same dislocation. Drying may have the same effect. Before or after carbon adhesion, fixation by such agents as alcohol, formalin, or mercuric chloride abolishes the specific localization.

The method shows lymph node stroma as a network of narrow ribbons which broaden out at intervals into macrophage bodies with recognizable, but indistinctly visible, negatively staining nuclei (Fig. 3).

In the 80–100-g rat, the follicular mounds exhibit no distinctive stromal morphology in the sites where primary follicular lymphocyte condensations could normally be

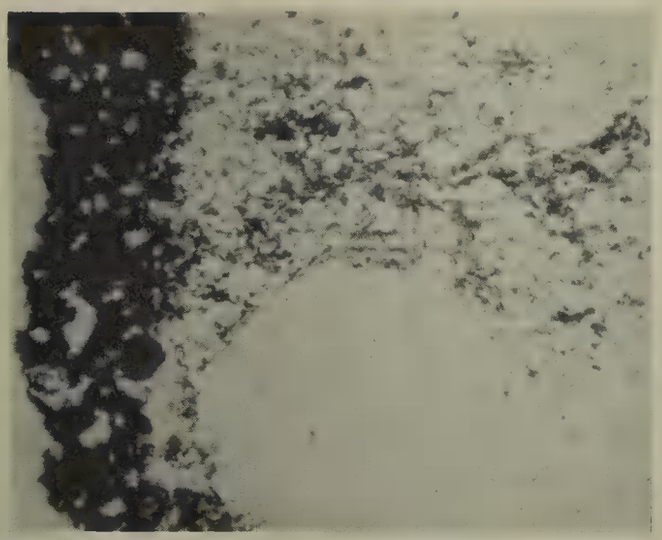

Fig. 6. Rat lymph node cortex, 2·5 h after carbon injection of corresponding footpad. Note how the carbon has penetrated the inner wall of the heavily marked subcapsular sinus (see left) and is spreading through the cortex along the arms of dendritic macrophages. Carbon does not penetrate into the circularly outlined primary follicle. 10μ frozen section. (×*c.* 170)

expected (Fig. 4). Even under mass antigen stimulation, there is no stromal counterpart of the blast cell circles which appear so early and consistently under these conditions. About the fifth and final day of the antigen course, and especially during the week following, follicular organization does appear, in the form of circular clusters of thickened macrophage processes (Fig. 5). It must be emphasized that this is a late event, only produced by intense and prolonged antigen stimulation.

Flow patterns of afferent lymph-borne carbon and flagellar antigen in the lymphoid cortex. Stromal follicles can thus be produced by antigen, but the evidence so far does not prove whether such follicles constitute a pre-formed structure in the unstimulated cortex. Studies of the early localization of lymph-borne carbon and flagellar antigen do, however, provide pointers in this direction.

0·2 c.c. 'Pelikan' indian ink is injected into the right hind footpad of the 80–100 g rat which has received no previous antigen. Two and a half hours later, the right para-aortic node is cut into frozen sections. These show the whole sinus system of the node outlined in black, and some direct penetration of the cortex by the carbon through the inner wall of the subcapsular sinus. Both these observations agree with Drinker *et al.*[3].

As the carbon penetrates the outer cortex, it outlines the processes of dendritic macrophages. It also negatively outlines circular follicular areas beneath the subcapsular sinus (Fig. 6). Nuclear counterstaining is possible, as carbon, adherent to dendritic macrophages *in vivo*, resists subsequent water dislocation in frozen sections. Such counterstaining shows that the area of minimum penetration corresponds with primary lymphocytic follicles.

Further information has been supplied by Nossal *et al.*[8], who have recently investigated the cortical distribution of afferent lymph-borne flagellar antigen marked by radio-iodine. Within 24 h of footpad injection of primary antigen, the corresponding popliteal lymph node shows circular or ovoid areas of tagged protein, which in terms of their contained lymphoid population may be indistinguishable from the rest of the cortex. At a later stage, blast cells are seen to appear and become numerous in each protein area, a classical lymphoid germinal centre being the end product.

Radio-iodine does not give sufficiently sharp localization of the actual cells involved in this protein localization. However, fluorescent antigen marking[5] has since shown that these cortical areas are composed of circular clusters of protein-marked dendritic macrophages identical in morphology to the antigen-induced follicles demonstrated by carbon adhesion, and presumably representing the same structure.

Conclusion. The combined weight of this evidence supports the idea of a pre-existent stromal macrophage centron, with a contained lymphoid population varying from small lymphocytes to blast cells according to the conditions of antigen stimulation. The problem is how to define this structure anatomically.

The difficulty is that the macrophages inside this structure do not appear to differ morphologically from the cortical macrophages outside, nor are they arranged in any constant or obvious way as a boundary structure.

Yet such a boundary seems very likely, whether in terms of the processes of macrophages, or in terms of some type of basement membrane. If such a barrier does exist, particles carried by lymph through the cortex[5] would be deflected from the outside of the stromal sphere (short-term carbon) or concentrated in the inside (antigenic protein), depending on complex physical factors such as barrier porosity, speed of lymph flow, and size of the particle concerned.

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IMMUNOLOGY OF THE MOUSE MAMMARY TUMOUR VIRUS (MTV): A QUALITATIVE *in vitro* ASSAY FOR MTV

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EXPERIMENTAL investigations of MTV are considerably hampered by the absence of a short-term *in vitro* assay. The conventional assay for the presence of infectious MTV has been the measurement of tumour development in susceptible test mice injected while young with extracts of infected mammary tissues or of milk[1]. This test requires 1–2 years for its completion. This time-interval has been shortened in a recently developed technique in which the end-point is the development of hyperplastic alveolar nodules in the mammary glands of test mice[2], but even this assay requires 4–5 months.

It is feasible to shorten the assay time still further by the development of techniques which measure the antigenicity rather than the biological activity of MTV. A number of attempts to demonstrate MTV by standard immunological techniques have been reported, using antisera obtained from rabbits or guinea-pigs immunized with MTV-containing tissue extracts or milk. It is well established that such antisera are capable of neutralizing the biological activity of MTV[3]. *In vitro* techniques have included passive haemagglutination, precipitin, and complement-fixation tests[4]. Precipitation reactions specific

for MTV in immunodiffusion agar plates have also been reported[5].

This article reports the development of the immunodiffusion technique as a qualitative assay for the presence of MTV. In its present form, the assay requires six weeks for its completion, including the time necessary for the preparation of the rabbit antiserum.

The methods used in the preparation of the tissue extracts and of the rabbit antisera were similar to those previously reported for work on the neutralization of MTV infectivity[1]. The tissues were homogenized in saline and clarified by a 30-min centrifugation at 1,500*g*. The supernatant was then centrifuged at 30,000*g* for 90 min. The resulting pellet was resuspended in saline, so that 1 ml. saline contained the material obtained from either 1 or 2 g, wet weight, of tissue. This suspension was clarified by centrifugation at 1,500*g* for 15 min, and the supernatant was frozen at 4° C until needed. Material prepared by this method was used as the antigen preparation for injection into rabbits, for absorption of the rabbit antisera, and in the immunodiffusion plates.

Young adult female New Zealand rabbits were used for the production of antiserum. Each rabbit received a total of tissue extract equivalent to 2·5–4·0 g of original tissue, in 4 weekly intramuscular injections, and was bled one week after the final injection. The resulting sera were frozen at 4° C until used. The antiserum from each rabbit was stored and used separately. Each antiserum was used undiluted in the plates except in the absorption experiments. Antisera were obtained from rabbits which had been immunized with one of the following tissues:

(*A*) Tissues obtained from isogenic *BALB/cCrgl* and *BALB/cfC3H/Crgl* mice.

(1) Lactating mammary glands from *BALB/c* females. These mice are not infected with MTV, and antisera from rabbits immunized with preparations of this material do not neutralize the biological activity of MTV[1].

(2) Transplant generations 3 through 7 of a *BALB/c* mammary tumour which arose spontaneously in a 21-month-old *BALB/c* female. No virus particles were observed when samples of the tumour were examined in the electron microscope[6].

(3) Lactating mammary glands from *BALB/cfC3H* females. This strain, in its 3rd to 5th generations, was started by fostering new-born *BALB/c* mice on *C3H/Crgl* lactating females, and using the babies when adult as generation 1 of the strain. Thus, these mice are isogenic with *BALB/c* mice, but are infected with MTV.

(4) Transplanted outgrowths of hyperplastic alveolar nodules and transplanted mammary tumours obtained from *BALB/cfC3H* females. These tissues contain virus particles as observed in the electron microscope and also biologically active MTV.

(*B*) Tissue obtained from a strain of mice which is not infected with MTV.

(1) Lactating mammary glands from *C57BL/Crgl* females.

(*C*) Tissues obtained from strains of mice which are infected with MTV.

(1) Lactating mammary glands from *C3H* females.

(2) Spontaneous and transplanted mammary tumours from *C3H* females.

(3) Spontaneous mammary tumours from *R*III/*Crgl* females.

(4) Spontaneous mammary tumours from *A/Crgl* females.

(5) Spontaneous mammary tumours from *DBA/2Crgl* females.

The tissue preparations routinely used as antigen in the immunodiffusion plates were obtained from *BALB/cfC3H* spontaneous and transplanted mammary tumours. In some experiments, tissue preparations of *DBA/2* mammary tumours were also used as the antigen. Preparations of mammary tumours from the other strains of mice (*C3H*, *R*III and *A*) have so far not been used successfully as antigens in the immunodiffusion plates.

In absorption experiments, the various antisera were absorbed with MTV-free or MTV-containing tissue preparations. The antisera were combined with the tissue extracts and then maintained at room temperature for 2 h with occasional manual agitation. The mixtures were clarified by centrifugation at 1,500*g* for 15 min, and the serum-containing supernatants were then added to the immunodiffusion plates.

The agar plates were prepared with 0·6 per cent Noble agar, 0·85 per cent sodium chloride, and 1 : 10,000 merthiolate in distilled water, at *p*H 7. Wells were cut with a 6-mm diameter cork borer in the pattern illustrated in Fig. 1. The distance from the centre of the antigen well to the centre of the antiserum well was 11 mm. Six antisera could be tested on each plate, and the experiments were designed so that the plates contained both a positive and a negative control. The standard antigen preparation was placed in the two centre wells one day before the antisera were added to the other wells. The centre wells were refilled with saline once daily for two days after the introduction of the antigen preparation. The plates were maintained at room temperature. Precipitate lines were visible within a few days, but the plates were routinely examined for a period of two weeks.

Each of the antisera already described here was tested against the standard MTV-containing antigen preparation in several separate experiments. In most experiments, no absorption of antiserum was carried out, and each antiserum produced several precipitate lines with the antigen. This is an expected result, since the standard antigen preparation contains not only MTV or MTV-induced antigen but also normal tissue antigens. The precipitate line characteristic of MTV was initially found by a comparison of the precipitate lines made by antisera obtained from rabbits immunized with *BALB/cfC3H* tissues (which contain MTV) with those made by antisera obtained from rabbits immunized with the isogenic but virus-free *BALB/c* tissues. Precipitate lines specific for

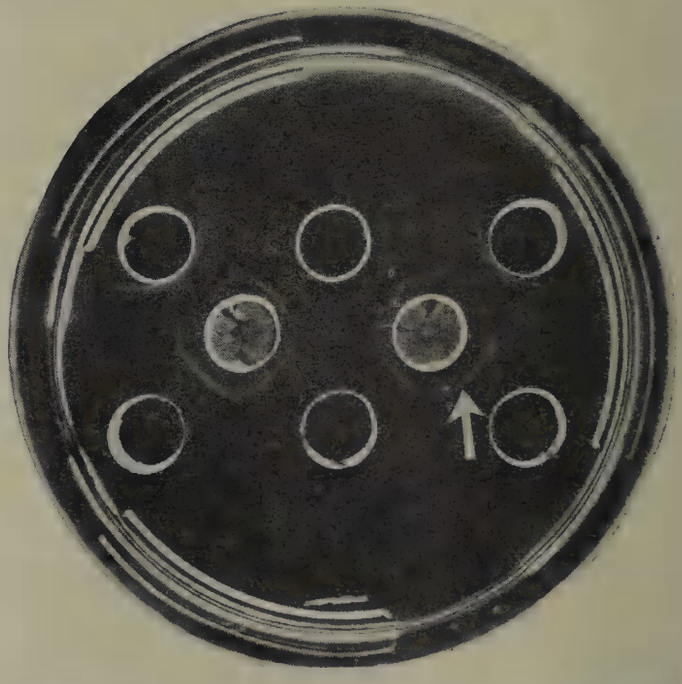

Fig. 1. Immunodiffusion of rabbit antisera and extracts of *BALB/cfC3H* mammary tumours. The three top wells were filled with, left to right, (1) rabbit antiserum against *BALB/c* lactating gland, (2) rabbit antiserum against *BALB/cfC3H* mammary tumour, and (3) rabbit antiserum against *BALB/c* lactating gland. The two centre antigen wells were filled with the standard antigen preparation of *BALB/cfC3H* mammary tumour. The three bottom wells were filled with, left to right, (1) rabbit antiserum against *C3H* mammary tumour, (2) rabbit antiserum against *DBA/2* mammary tumour, and (3) rabbit antiserum against *C3H* mammary tumour. The characteristic precipitate lines (indicated by the arrow) are found near the antigen wells wherever the advancing antiserum contained antibodies against MTV, but not in the two areas where the advancing antisera were from rabbits immunized with virus-free *BALB/c* tissues (× 1·3)

tissue antigens were observed in the vicinity of the antiserum wells; the precipitate line characteristic of MTV was found near the antigen wells (Fig. 1). The MTV specificity of the line was tested by using antisera from rabbits immunized with tissues from other strains of mice known to contain MTV. The specificity was further tested by using antisera which had been absorbed with virus-free tissue preparations; the absorbed antisera produced only the line characteristic of MTV in the immunodiffusion plates.

As recorded in Table 1, all the antisera from rabbits immunized with normal or neoplastic tissues containing MTV (obtained from 5 different strains of mice) produced the characteristic precipitate line. On the other hand, none of the antisera from rabbits immunized with *BALB/c* or *C57BL* tissues (which do not contain MTV) produced this line.

Further, in the absorption experiments completed so far (Table 2), extracts of tissues which do not contain MTV were not capable of absorbing the antibodies responsible for the formation of the characteristic precipitate line. In contrast, the antibodies were removed by absorption with preparations of either normal or neoplastic tissues which do contain MTV.

The fact that antisera from rabbits immunized with tissues obtained from a variety of mouse strains infected with MTV all produced the characteristic precipitate line in this test system indicates that the MTVs carried by different strains of mice contain at least one antigen in common. This is in agreement with the results of previous neutralization studies, in which antisera from rabbits immunized with the MTV from one strain of mice were capable of neutralizing the biological activity of the MTV from other strains of mice[1,7]. Based on this common antigenicity, the immunodiffusion technique has a potential usefulness as an assay system for the MTV from any strain.

Not only similarities but also differences in biological activity[8], morphology[9], and antigenicity[10] of the MTVs from various strains of mice have been reported. The scope of such investigations has been limited by the cumbersome *in vivo* assay for the presence of infectious MTV. Moreover, there frequently is little correlation between the presence of the type *B* virus particles considered to be MTV and the presence of biologically active MTV, and various explanations for this lack of correlation have been proposed[11]. An assay method which is based on the presence of a common MTV antigenicity, rather than on biological activity, can be expected to resolve some of these differences in interpretation.

Tabie 1. Production of Characteristic MTV Precipitate Line by Various Rabbit Antisera tested in Immunodiffusion Plates against Tissue Extracts of *BALB/cfC3H* Mammary Tumours

Tissues used for immunization of rabbits	No. of rabbits	No. of sera producing characteristic precipitate line
(1) Tissues containing no MTV:		
BALB/c lactating glands	5	0
BALB/c mammary tumours	1	0
C57BL lactating glands	2	0
(2) Tissues containing MTV:		
BALB/cfC3H lactating glands	4	4
BALB/cfC3H nodule outgrowths and mammary tumours	5	5
C3H lactating glands	2	2
C3H mammary tumours	3	3
*R*III mammary tumours	1	1
A mammary tumours	2	2
DBA/2 mammary tumours	2	2

Table 2. Production of Characteristic MTV Precipitate Line by Antisera from Rabbits immunized with MTV-containing Tissue Extracts, after Absorption of the Sera with various Tissue Preparations

The antisera were tested in immunodiffusion plates against tissue extracts of *BALB/cfC3H* mammary tumours

Tissues used for absorption	Antisera from rabbits immunized with *BALB/cfC3H* tumour	Antisera from rabbits immunized with *C3H* tumour
1) Tissues containing no MTV:		
BALB/c lactating glands	+	+
BALB/c mammary tumours	+	+
(2) Tissues containing MTV:		
BALB/cfC3H lactating glands	0	0
BALB/cfC3H mammary tumours	0	0

+, Characteristic precipitate line formed; 0, no characteristic precipitate line.

The antigenic component responsible for the production of the characteristic precipitate in the immunodiffusion assay appears to be the intact type *B* virus particle[12]. This particulate antigenic component can be sedimented by centrifugation at 30,000*g* for 1·5 h or less. The MTV-associated antigen found by Lezhneva[5] in the lactating mammary glands of *R*III mice was a much smaller component. It remained in the supernatant even after centrifugation at 100,000*g* for 1·5 h. These two MTV-associated components may represent the two sizes of infective MTV reported by Moore[13]. It is also quite possible that the smaller component may represent fragments of the type *B* particle, or a virus-induced antigen.

As reported here, all MTV-containing antigen preparations tested were successfully used for the production of antisera in rabbits which then produced the characteristic precipitate line when tested against the standard MTV-containing antigen preparation in the immunodiffusion plates. However, some of the same preparations did not produce the characteristic precipitate line when they were used as antigens in the plates, and tested against standard antisera. This is probably related to the concentration of MTV antigen in these preparations. The concentration of the type *B* virus particle varies considerably in MTV-containing tissues from different sources[9]. As expected, MTV-free *BALB/c* tissue preparations cannot be used as antigen in immunodiffusion.

The qualitative *in vitro* test described herein is, nevertheless, ideally suited to indicate the presence or absence of MTV in tissues, by using the suspect tissues as antigen for immunization of rabbits and then testing these antisera against standard MTV-containing preparations in the immunodiffusion plates. The assay can also be developed to provide data on the relative amounts of MTV-related antigen in various tissues, by means of absorption of standard antisera with the different tissue preparations. Antigenic differences between the MTVs carried by different strains can also be examined, again utilizing absorption experiments. That such experiments may be subject to quantitation is indicated by work with other viruses[14]. Quantitative absorption experiments designed to investigate these possibilities are currently in progress.

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INCREASED SENSITIVITY OF *IN VITRO* MURINE LEUKAEMIA CELLS TO FRACTIONATED X-RAYS AND FAST NEUTRONS

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FOLLOWING the original report of Elkind and Sutton[1], the variation with time in radiosensitivity after a single dose of radiation has been studied for a number of different cell systems. They have all shown a similar pattern of change, but with differences in the degree of variation. In no case, however, has the net effect of a given dose delivered in two fractions been greater than that of a similar undivided exposure.

In this article, investigations are reported using a mammalian cell suspension in which it has been found that the radiosensitivity increases above normal following an initial dose of radiation. The data are described and discussed in relation to possible mechanisms operating.

Murine leukaemia cells growing *in vitro* in suspension were used for the investigations. These were made available by Dr. Peter Alexander of the Chester Beatty Research Institute[2]. The cells were grown at 37° C in a specially prepared, balanced medium[3], and under control conditions have a doubling time of 9–10 h. The cells were irradiated in polypropylene tubes at 37° C with 230 kVp. X-rays (half value layer, 1·5 mm copper) at a dose rate of 41 rads/min, or with fast neutrons having a mean energy of 6 MeV at about 50 rads/min. The dose-survival curves were obtained by backward extrapolation of growth curves, following varying doses of radiation. Since the growth curves were parallel for all doses approximately 48–60 h after radiation, extrapolation gave the surviving fraction after a correction was made for mitotic delay[4]. This correction is not large, and resulted in an uncorrected D_{37} of 55 rads becoming 62 rads on correction.

In order to evaluate the survival kinetics of the cells in the divided dose experiments, cell survival 100 h after the initial exposure was measured. Survival at this time can be considered a measure of reproductive integrity since, even accounting for mitotic delay, 100 h is equivalent to approximately 10 cell cycles. Radiation doses totalling 200 rads of X-rays or 80 rads of neutrons were used for all experiments. Counts of viable cells were made in standard haemocytometers using phase-contrast microscopy.

Fig. 1 shows dose survival curves for X-rays and fast neutrons respectively. The X-ray curve clearly has no very substantial shoulder, but the scatter of the experimental points does not allow the degree of shoulder to be determined with any precision. Alexander and Mikulski[2], using the same cells with the same medium, observed a slight shoulder, but a correction for mitotic delay would very largely eliminate it. With the neutrons there would appear to be very little, if any, shoulder. With neutrons the D_{37} is 27 rads; this implies a relative biological effectiveness (RBE) of 2·3, when compared with the D_{37} of 62 rads for 250 kVp. X-rays.

Fig. 2 shows the mitotic delay induced by a range of doses of X-rays. Preliminary measurements have also been made with neutrons, for which the response shows a similar pattern of change with time as for X-rays, with an RBE of 2–2·5.

Fig. 3 shows the relative survival after a pair of equal exposures for varying intervals of time between them. It can be seen that with both X-rays and neutrons there is a brief period of decreased sensitivity to a second exposure, followed by an interval (from about the fourth to tenth hour) in which sensitivity is actually increased above normal. The form of the curve is similar for the two types of radiation with smaller variation in the case of neutrons. With X-rays, and an interval of about 6 h between exposures, survival is approximately 50 per cent of that for cells exposed to the same total amount of radiation at one time, and with neutrons about 70 per cent.

Using X-rays, an investigation was also made of survival when the time-interval between radiation fractions was kept constant at 5 h and the exposure fractions varied. The relative survival curve is given in Fig. 4, and it shows an increased sensitivity for all tested fractionations with the maximum effect when the initial dose was 20 rads and the second dose 180 rads.

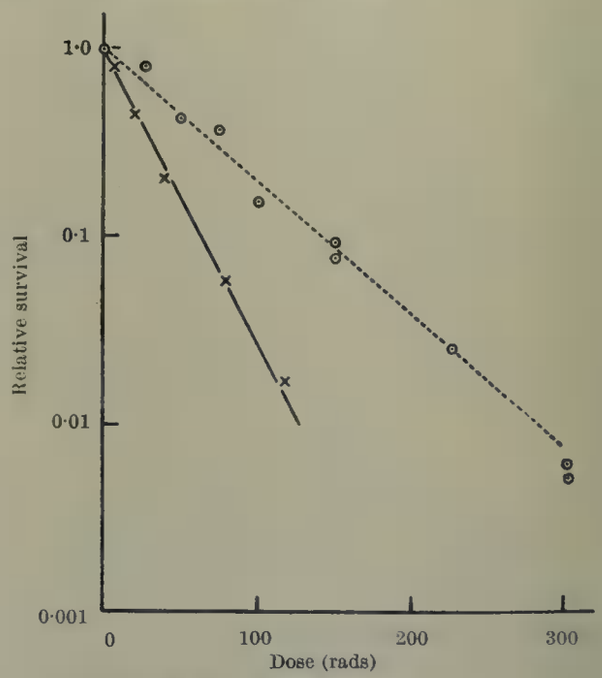

Fig. 1. Dose-survival curves for X-rays and neutrons. ○, X-rays; ×, neutrons

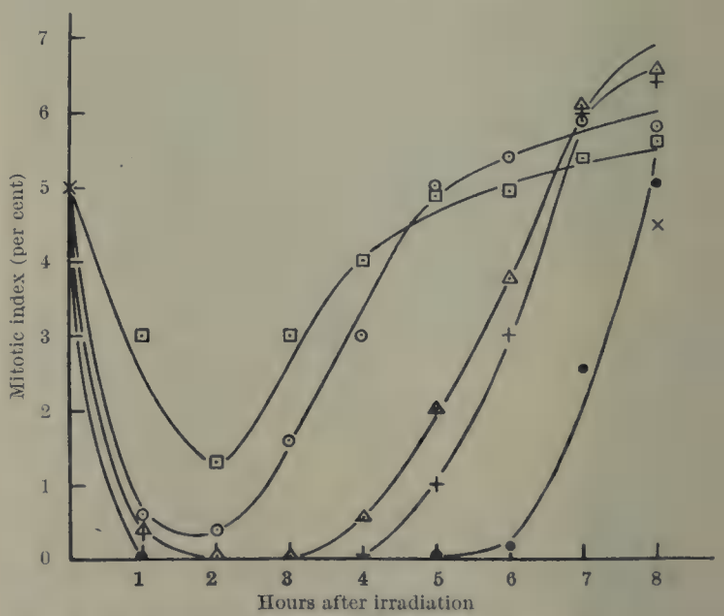

Fig. 2. Mitotic index curves following different doses of X-radiation (rads). ⊡, 20; ⊙, 25; △, 50; + 100; ●, 200

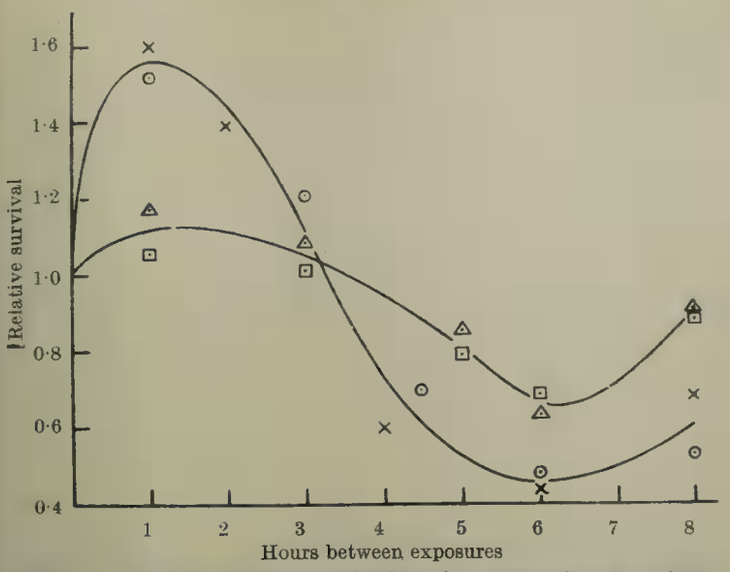

Fig. 3. Cell survival following a pair of equal exposures given at various intervals. Two doses each of 100 rads given for X-rays, and two doses each of 40 rads for neutrons. Points represent results from two separate experiments. △, □, Neutrons (2 × 40 rads); ×, ⊙, X-rays (2 × 100 rads)

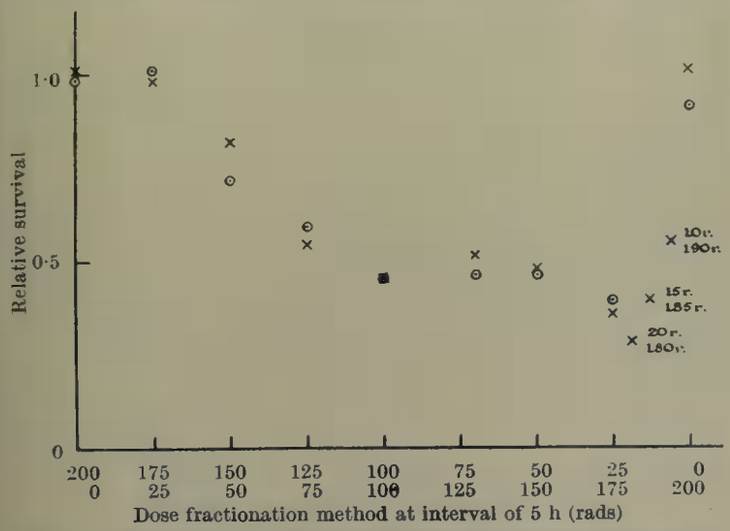

Fig. 4. Cell survival under X-rays following different fractionation schemes at a constant time interval of 5 h, compared with survival following a single exposure of 200 rads. Points represent results from two separate experiments

The two features of particular interest in these results are: (1) that the sensitivity after an initial conditioning dose is actually increased above normal; (2) that with an interval of 5 h between exposures the effect of a total dose of 200 rads is greater when the smaller fraction is given first (the maximum effect on survival being achieved with an initial dose of 20 rads).

In other cell systems in which the response to divided doses has been reported, the increase in sensitivity following the initial decrease has been relative only, sensitivity to the second exposure approaching, but not exceeding, control-levels. However, the dose-survival curves for these systems all have a substantial initial shoulder and therefore it is possible that at least part of the explanation for the difference lies in the lack of a significant initial shoulder in the survival curve of the cells used in the present study.

Elkind[5] originally concluded that recovery after an initial exposure was associated with reconstruction of the shoulder of the dose-survival curve; that is, with return of the extrapolation number, changed by the conditioning exposure, to its original value. Afterwards, the work of Terasima and Tolmach[6] gave support to Kallman's hypothesis[7] that the change in sensitivity was related to a shift in the proportion of cells in different compartments of the mitotic cycle characterized by different sensitivities. Recently, synchronization investigations[8,9] provide data supporting the model of recovery kinetics for dose fractionation experiments which had been suggested by Elkind, Sutton and Moses[10]; that is, that the initial decrease in sensitivity following a conditioning exposure results from recovery of sub-lethal damage (reconstitution of the shoulder of the dose-survival curve) while the subsequent relative increase in sensitivity is the result of phasing of cells within sensitive phases of the mitotic cycle.

At the same time one must recognize that other processes may also be operative. It is of interest that Beer *et al.*[11] and Lett *et al.*[12] have shown that various factors connected with culture conditions can alter the radiosensitivity of these leukaemia cells, neither D_{37} nor the extrapolation number remaining constant. It is possible that when these cells are given two radiation exposures, the first exposure may modify the growth conditions so as to alter the response to the second exposure.

If the difference between the present results and those of Till and McCulloch[13], for example, were a consequence of the initial shoulder, then subtraction of the two curves should show the effect of the shoulder. Fig. 5 shows the results of this operation. Making allowance for difference in time intervals (as indicated), subtraction of the leukaemia curve from that obtained by Till and McCulloch for mouse bone marrow cells yields the curve (*c*), which could possibly represent the 'shoulder' effect. On this basis, the variation in sensitivity found for the leukaemia cells would have to be due to variation in radiation response with cell phase, associated with partial synchronization of the cells following irradiation, or to an entirely new process not so far postulated.

One conclusion that can be drawn from the mitotic index data of Fig. 2 is that the variation of sensitivity following a conditioning dose is not the result of an altered sensitivity during mitosis. So far as phasing effects are concerned, interpretation of the mitotic data is not straightforward since, particularly with the higher doses, many of the cells being counted, including those in mitosis, are 'doomed' (destined to die in a future division cycle). Phasing of the viable cells might be obscured by the presence of 'doomed' cells, in which case, with passage of time, peaks of mitotic activity of viable cells should be detectable, as the 'doomed' cell population diminished, provided the variation in post-irradiation cell-cycle times was not sufficient to obscure such peaks. Preliminary explorations for such peaks in leukaemia cells have been fruitless.

The significance of cell phasing in divided-dose survival kinetics in any cell system still remains to be clarified. Experimental investigations have given very diverse results concerning the change of sensitivity with phase of the cell cycle. A further difficulty in explaining the results by a simple phasing effect is that Whitmore[8] found, in

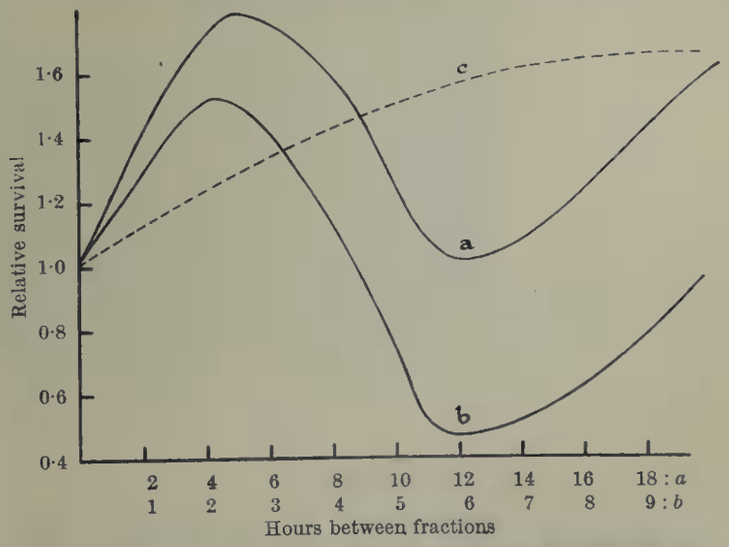

Fig. 5. Comparison of a typical curve (*a*) of change in sensitivity to a second exposure following a conditioning exposure (ref. 13) with the curve (*b*) obtained for leukaemia cells. Differences in time between fractions for the two curves are as indicated. Curve (*c*) results from a subtraction of curves (*a*) and (*b*)

his synchronized *L* cells, an initial upswing in the 'recovery curve' following a conditioning exposure during all phases of the mitotic cycle. Apparently unrelated to phasing, this initial upswing was thought to be primarily the result of recovery of that sub-lethal damage which gives rise to the shoulder of the curve. However, since this upswing is also seen in the leukaemia cells with both X-rays and neutrons where the shoulder is either small or absent (Fig. 3), it seems necessary to postulate some other process.

There is, however, another possible interpretation of the results which does not require the assumption of a new process of recovery. If the sensitivity varies over the various phases of the cell cycle, the survival curve for the leukaemia cells cannot be a true exponential, but must represent the combined survival of various fractions. It is possible that one or more of these fractions has an initial shoulder. As a simple model it can be shown, for example, that if the cells consisted of 60 per cent with a D_{37} of 62 rads and an extrapolation number of 1·67 and 40 per cent with a D_{37} of 20 rads and an extrapolation number of 1·00, the combined curve would be practically indistinguishable from an experimental curve with a D_{37} of 62 rads. In this case the component with a shoulder could be responsible for the increased survival at 1 h and the subsequent swing could be due to phasing. The finding that the effect of the split dose was less with the larger fraction given first would be explained if the selective killing by the conditioning exposure were such that, at 5 h, a greater proportion of surviving cells were concentrated in their sensitive phase after a small dose than after a large dose. The maximal effect found with about 20 rads as the initial exposure presumably represents the optimal combination of 'sensitization' and size of the second exposure.

To decide whether the foregoing hypothesis is correct, or whether some other mechanism, such as modification of actual repair processes, is operating requires investigation of the effect of different dose fractions at different time-intervals and of the response of synchronized populations of the cells.

Studies with high LET radiation could presumably help a great deal in disentangling the various mechanisms Operating. Radiation of sufficiently high LET might give a true exponential survival curve instead of a synthetic one, and in these circumstances it is generally assumed that no recovery would be expected[14]. Fast neutrons give rise to a wide spectrum of LET, and the reduced variation in sensitivity found with neutrons, as compared with the lower LET X-rays, is consistent with this idea. On the other hand, the possibility must not be ignored that some recovery processes may operate even with high LET radiation.

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EFFECT OF GASTRIN ON ISOLATED SMOOTH MUSCLE PREPARATIONS

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EDKINS[1,2] found that intravenous injections of crude aqueous antral extracts into anaesthetized cats stimulated the secretion of acid gastric juice. Since then many workers have studied the secretory activity of antral extracts. Blair *et al.*[3] noticed that the intravenous injection of an antral extract into anaesthetized cats not only stimulated gastric secretion, but also increased the tone of the stomach and small intestine. Gregory and Tracy[4,5] extracted hog antra and obtained two pure, almost identical, peptides which they called gastrins I and II. These stimulated gastric secretion and also contracted the stomach and jejunum in the dog. Very little is known about this stimulation of gastro-intestinal motility and the present experiments were carried out to examine the effect of pure gastrin II on guinea-pig and rat smooth muscle preparations.

Rat colon and rat uterus preparations were suspended in an organ bath containing de Jalon's fluid at 28° C bubbled with oxygen. All the other smooth muscle preparations were suspended in Krebs's solution at 37° C bubbled with 95 per cent oxygen and 5 per cent carbon dioxide. The responses of the tissues were recorded on a smoked drum by a light frontal writing lever (magnification × 12). In some experiments guinea-pig ileum preparations were stimulated transmurally[6] through coaxial electrodes. Drugs used were acetylcholine perchlorate, cocaine hydrochloride, dimethylphenylpiperazinium iodide (DMPP), hexamethonium bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), (−) hyoscine hydrobromide, gastrin II, mepyramine maleate, methysergide, morphine sulphate and physostigmine sulphate. The concentrations of cocaine hydrochloride, DMPP and methysergide are expressed as the salt. Other concentrations are in terms of base.

Gastrin II (1–2 μg/ml.) caused small contractions of the rat fundus strip preparation[7], the rat duodenum and the rat colon. Guinea-pig sacculus rotundus and a strip of antrum and body cut from the rat stomach contracted feebly, and the rat uterus not at all, in the presence of 1–4 μg/ml. of gastrin. The most responsive preparation encountered was guinea-pig ileum which usually contracted well in response to 0·1–4 μg/ml. of gastrin. Misiewicz and Waller (personal communication) found that 0·5–1 μg/ml. of gastrin I contracted 5 of 11 strips of human isolated stomach and that tachyphylaxis occurred. The analysis of the action of gastrin described here was carried out on the guinea-pig isolated ileum preparation.

Gastrin II produced a contraction of guinea-pig ileum which was rapid in onset and which quickly reached a peak. Sometimes the contraction was maintained, but often the tissue partially relaxed while gastrin was still

in the bath. A second dose added soon after the first had been washed out produced a smaller effect. In order to prevent this tachyphylaxis, gastrin was administered at intervals of approximately 15 min. Mepyramine (0·4–100 ng/ml.) completely blocked the responses to histamine; it had no effect on the responses to gastrin in 7 experiments, but in another experiment 10 ng/ml. of mepyramine slightly reduced the effect of gastrin. The 5-HT antagonist methysergide (which only partially antagonizes 5-HT on the guinea-pig ileum[8]) had no effect on the responses to gastrin in 3 experiments (methysergide 10–100 ng/ml.). In another 4 experiments methysergide (100 ng/ml.) reduced the contraction produced by gastrin, but since the responses to acetylcholine were also a little reduced in 3 of these 4 experiments the antagonism was probably non-specific.

In each of 6 experiments hexamethonium (10 μg/ml.) almost completely abolished the response of the ileum to DMPP but had no effect on the response to gastrin (Fig. 1). Hyoscine (2–200 ng/ml.), however, completely abolished the contraction produced by acetylcholine (9 experiments) and either completely abolished the response to gastrin (7 experiments) or reduced its effect (2 experiments; hyoscine 4 ng/ml.). When the hyoscine was washed out the effects of acetylcholine and gastrin returned together (Fig. 2). During a hyoscine block, additions to the bath of 20–40 ng/ml. of eserine (3 experiments) restored or even potentiated the responses to acetylcholine and gastrin (Fig. 2). Eserine (20–100 ng/ml.) added to the bath in the absence of hyoscine potentiated the responses to acetylcholine and gastrin equally in 4 experiments, and potentiated gastrin more than acetylcholine in 2 experiments.

These results suggest that gastrin acts either on acetylcholine receptors in the muscle or, more likely, on the postganglionic parasympathetic nerves at a site not blocked by hexamethonium to cause the release of acetylcholine. These possibilities were tested by using: (*a*) morphine to prevent the release of acetylcholine[9]; (*b*) anoxia to prevent nervous conduction[8]; (*c*) cocaine in an attempt to anaesthetize the nervous tissue in the ileum.

The responses to gastrin and DMPP were reduced (3 experiments) or almost abolished (2 experiments, see Fig. 3) by morphine (10–100 ng/ml.). The responses to acetylcholine were slightly depressed in 3 of the 5 experiments, and the responses to transmural stimulation were reduced in each of 3 experiments in which coaxial stimulation was used.

The effects of anoxia on responses to gastrin were studied in 4 experiments by replacing the oxygen/carbon dioxide mixture with nitrogen or with a nitrogen/carbon dioxide mixture. Anoxia abolished the effects of transmural stimulation and almost completely prevented the responses to gastrin and DMPP (Fig. 4). The effects of acetylcholine and histamine were reduced to a much less extent and the responses of the ileum to histamine and acetylcholine approximated their pre-anoxia effects when the doses of the agonists were doubled. On the other hand, increasing the doses of gastrin and DMPP 7–10 times produced only moderate or small contractions. These results indicate that gastrin acts on nervous tissue, but since it produced a small effect on the anoxic ileum it is possible that gastrin also has a small direct effect on muscle cells. In each of 6 experiments cocaine hydrochloride (10–25 μg/ml.) almost completely blocked the effect of DMPP. In 2 of these experiments the responses to gastrin were unaffected, but in 3 experiments the effect of gastrin was slightly reduced (Fig. 5) and in one instance it was almost completely abolished. The effect of cocaine (20 μg/ml.) on the response of the ileum to transmural stimulation of the nerves by single shocks (6/min, 0·5 msec and 3 V) was examined in 3 of these 6 experiments. Surprisingly, the contractions were only slightly reduced in 2 experiments and were unaffected in the other, thus indicating that cocaine in this concentration has little or no effect on transmission in postganglionic parasympathetic nerve endings. The finding that cocaine always abolished the effect of DMPP, but only sometimes reduced the effect of gastrin, supports the evidence obtained with hexamethonium that DMPP and gastrin act at different sites. The variation in the effect of cocaine on the response to gastrin is apparently explained by the finding that cocaine only sometimes reduced the effect of electrical stimulation, presumably by partial anaesthesia of the nerves.

All these experiments indicate that gastrin acts on the postganglionic parasympathetic nerves, and suggest that in the guinea-pig ileum the contraction produced by gastrin is caused by the release of acetylcholine. Such a view is consistent with the results of Gregory and Tracy[5] that atropine reduces the motor effect of gastrin on the stomach and jejunum of the dog. It is of great interest that atropine also inhibits the stimulation of gastric secretion by gastrin in the dog[10–12] and in man[12]. It may follow that gastrin stimulates secretion by acting on nerves to release acetylcholine.

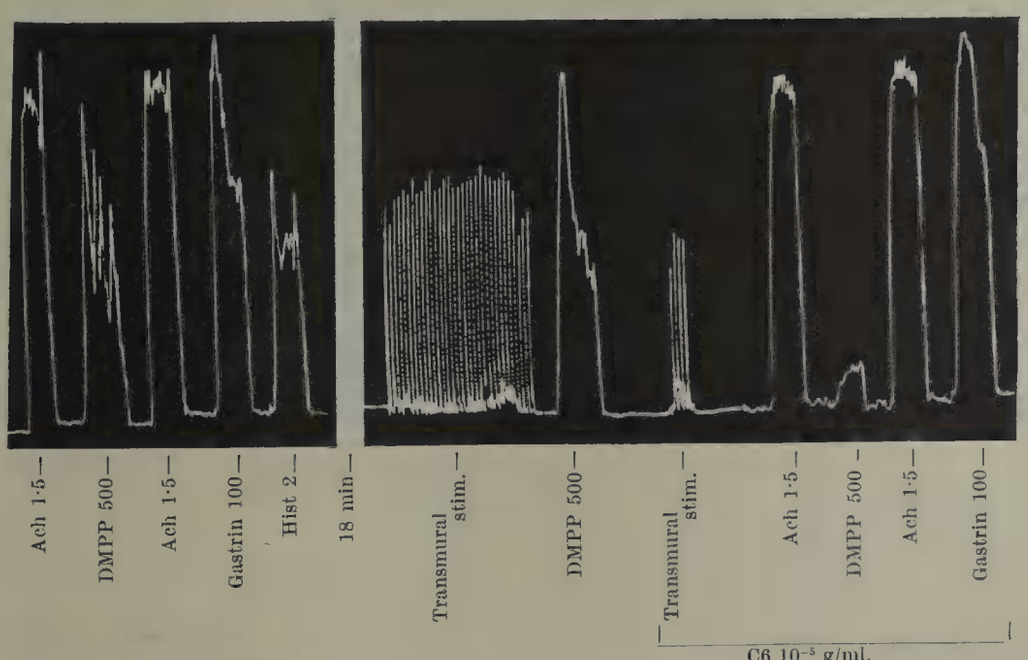

Fig. 1. The lack of effect of hexamethonium on the response of guinea-pig ileum to gastrin. Hexamethonium inhibited the response to DMPP but did not alter the response to gastrin. The numbers by the agonist drugs represent drug concentrations in ng/ml.

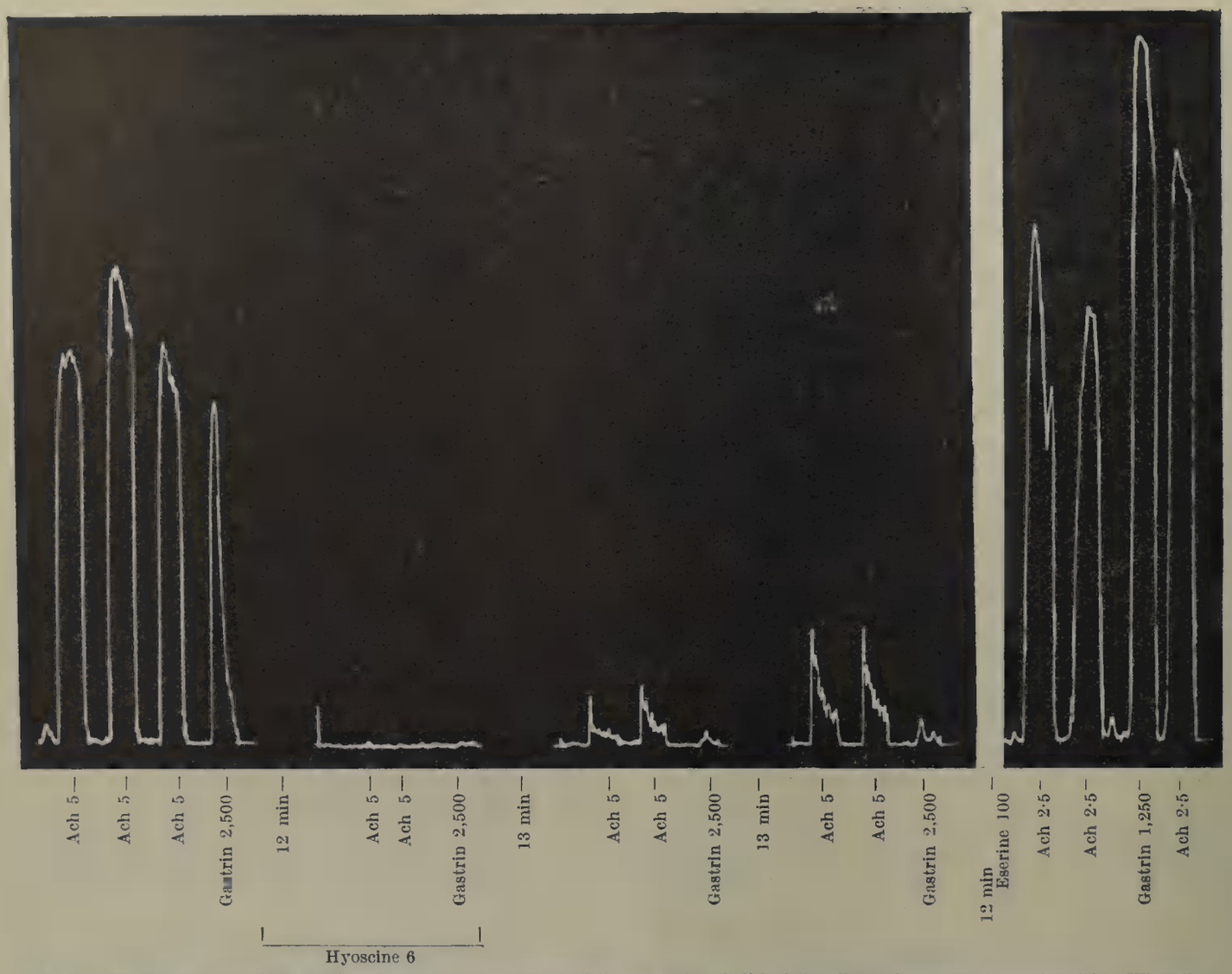

Fig. 2. The effects of hyoscine and of eserine on the response to gastrin. Hyoscine abolished the effects of acetylcholine and gastrin. When the hyoscine was washed out the responses to acetylcholine and gastrin started to return. After the addition of eserine both gastrin and acetylcholine were greatly potentiated. The numbers by the drugs represent concentrations in ng/ml.

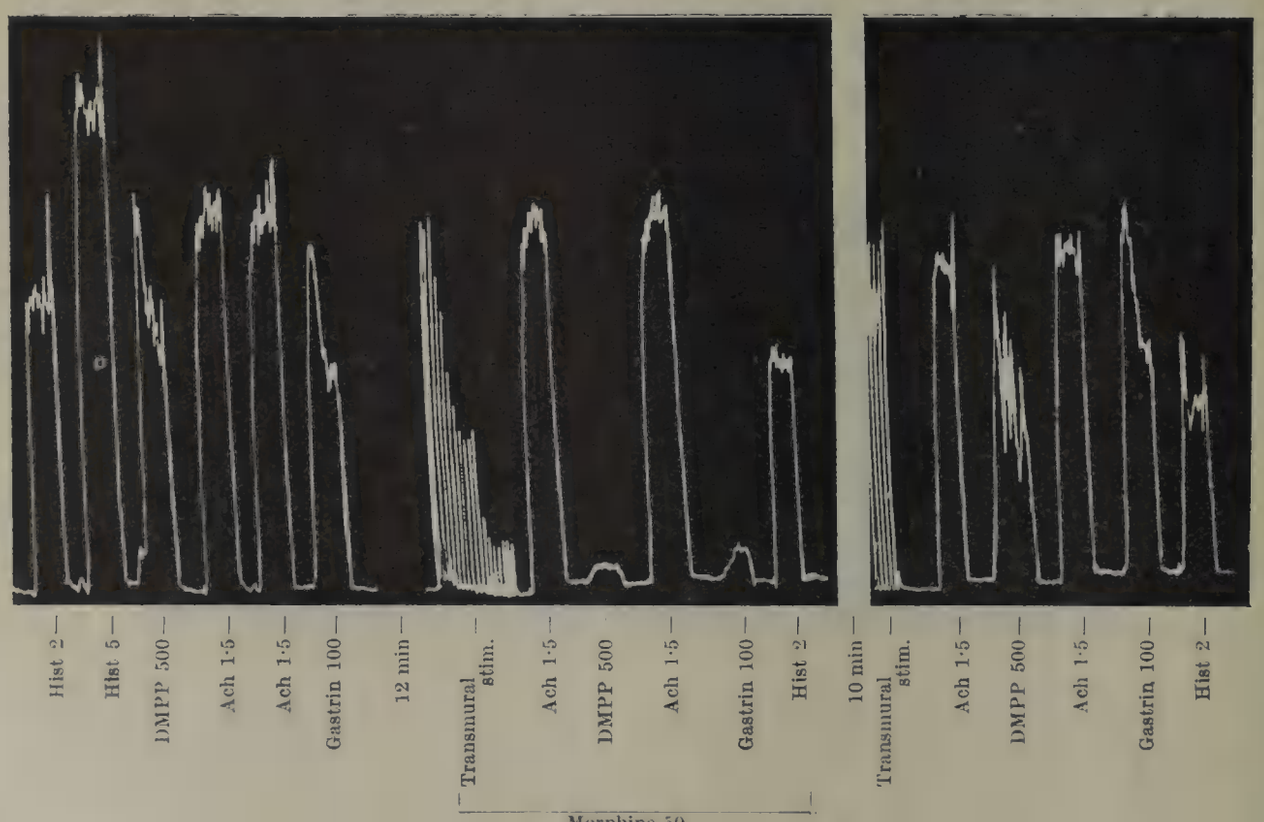

Fig. 3. The effect of morphine on the response to gastrin. Morphine greatly reduced the effects of gastrin, DMPP and transmural stimulation. The numbers represent drug concentrations in ng/ml. During transmural stimulation (3 V, 0·5 msec, 4 shocks/min) the drum speed was reduced to one-eighth

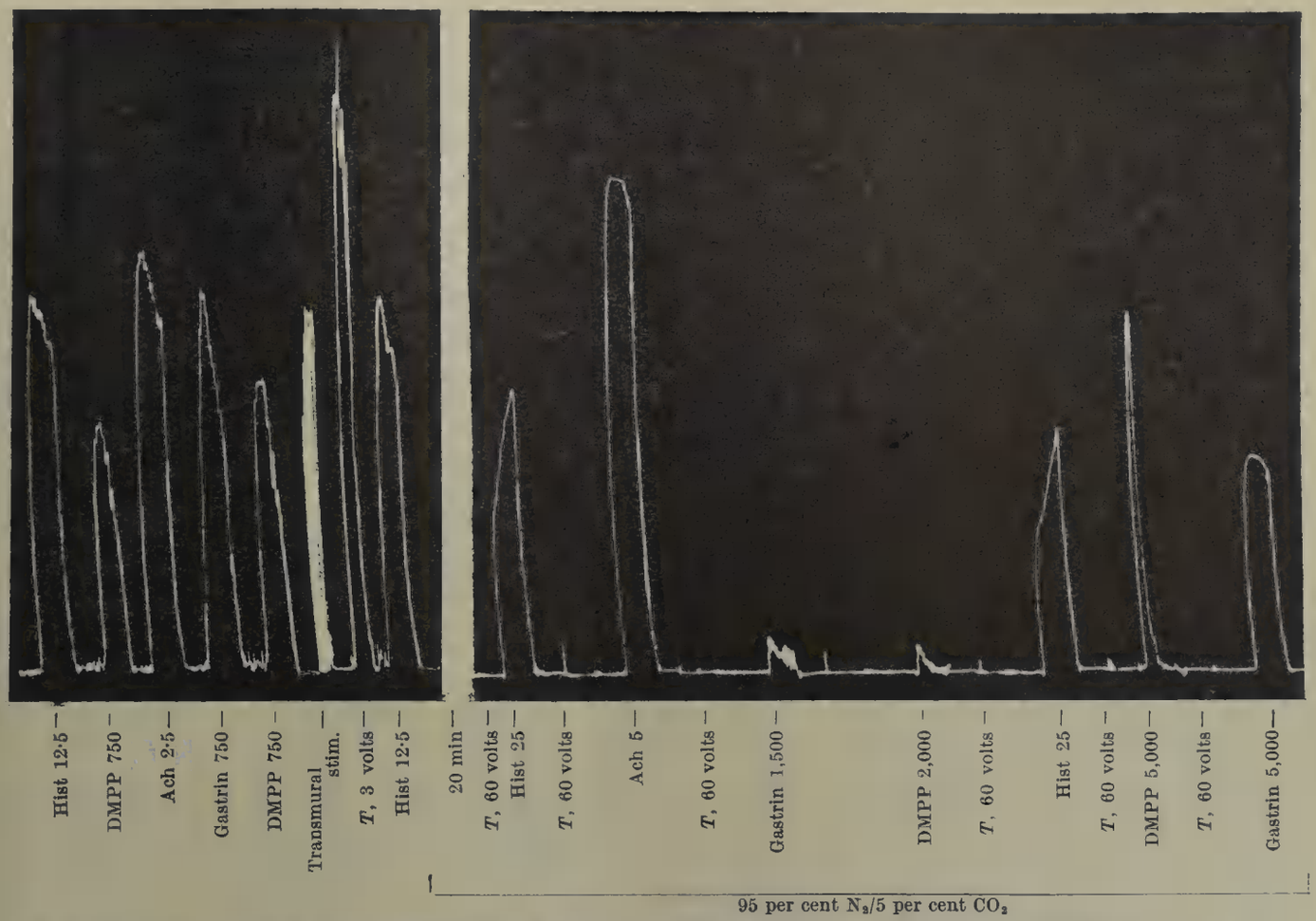

Fig. 4. The effect of anoxia on the response to gastrin. Anoxia completely abolished the effect of transmural stimulation at 3 V, 1 msec, 6 shocks/min; 3 V, 1 msec, 5 shocks/sec (*T*, 3 volts) and 60 V, 1 msec, 5 shocks/sec (*T*, 60 volts). Anoxia slightly reduced the responses to acetylcholine and histamine but greatly reduced those to gastrin and DMPP. The responses to gastrin and DMPP approached their pre-anoxia levels when the doses were increased from 750 ng/ml. to 5,000 ng/ml.

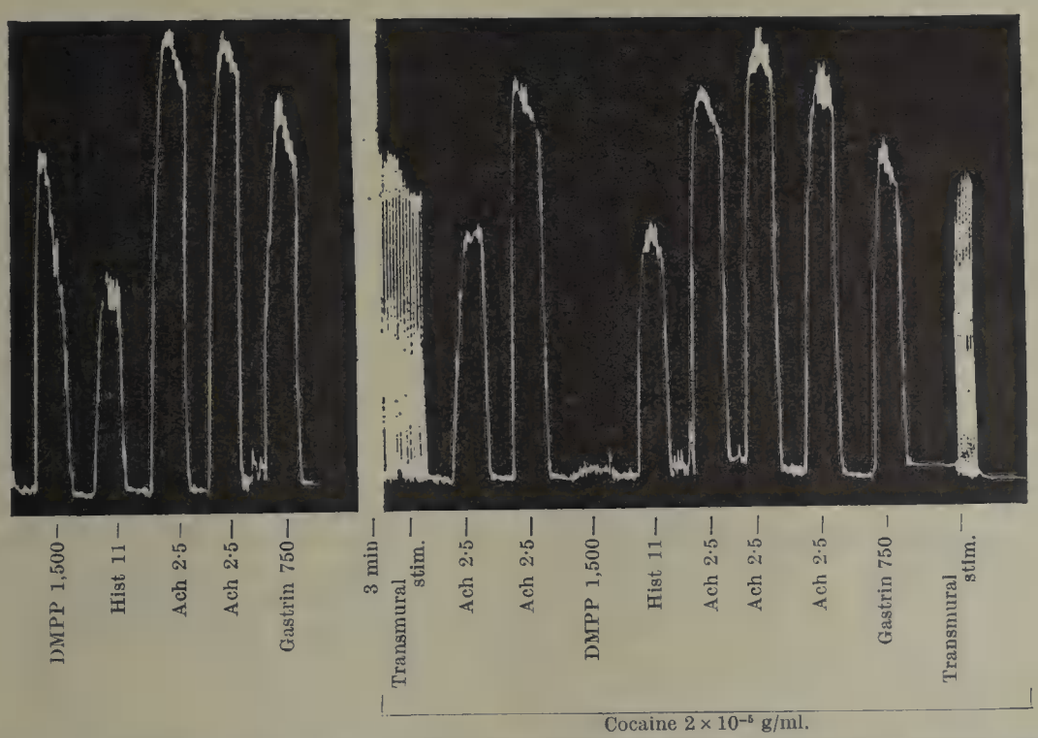

Fig. 5. The effect of cocaine on the response to gastrin. Cocaine almost abolished the response to DMPP but only slightly reduced the effects of transmural stimulation and of gastrin. The numbers by the agonists represent the drug concentrations in ng/ml. During transmural stimulation (3 V, 0·5 msec, 6 shocks/min) the drum speed was reduced to one-eighth

I thank Prof. R. A. Gregory and Dr. B. A. L. Hurn of the Wellcome Researeh Laboratories for samples of gastrin II, and Dr. J. R. Vane for his advice.

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LETTERS TO THE EDITOR

PHYSICS

Structural Basis of Neutron and Proton Magic Numbers in Atomic Nuclei

In 1933 Elsasser[1] pointed out that some of the properties of atomic nuclei correspond to greater stability for certain numbers of neutrons and protons (given the name magic numbers) than for other numbers; the magic numbers for both N (neutron number) and Z (proton number) are 2, 8, 20, 50, 82 and 126. (Less-pronounced effects are observed also for N or Z equal to 6, 14, 28, 40, and some larger numbers. The set of magic numbers is often assumed to include 28.)

The magic numbers do not have the values $(2n^2)$ for completed shells of fermions (with all states with total quantum number n, azimuthal quantum number $l \leqslant n-1$, occupied by pairs), which are 2, 8, 18, 32, 50, ..., nor the values for certain shells and sub-shells that lead to maximum stability for electrons in atoms, which are 2, 10, 18, 36, 54 and 86.

It was discovered by Mayer[2] and by Haxel, Jensen and Suess[3] that the magic numbers can be accounted for by use of the sub-sub-shells corresponding to spin-orbit coupling of individual nucleons; that is, to the values of $j=l+1/2$ and $l-1/2$ for the two sub-sub-shells of each sub-shell. For example, they[4] assign to N or $Z=50$ the configuration $(1s1/2)^2(1p3/2)^4(1p1/2)^2(1d5/2)^6(2s1/2)^2(1d3/2)^4(1f7/2)^8(2p3/2)^4(1f5/2)^6(2p1/2)^2(1g9/2)^{10}$, which may be written more briefly as $1s^21p^61d^{10}2s^22p^61f^{14}(1g9/2)^{10}$.

The evidence for spin-orbit coupling and for the Mayer–Jensen shell model is convincing. It is, however, difficult to understand, on the basis of their arguments, why the six magic numbers should be outstanding among the many numbers corresponding to the completion of spin-orbit sub-sub-shells, which (for the Mayer–Jensen sequence[4] of energy-levels) are 2, 6, 8, 14, 16, 20, 28, 32, 38, 40, 50, 56, 64, 68, 70, 82, 92, 100, 106, 110, 112, 126, 136, 142, ...

In the course of developing a theory of nuclear structure based on the assumption of closest packing of clusters of nucleons[5], I have found that the magic numbers have a very simple structural significance: 2 and 8 correspond to the closed shells $1s^2$ and $1s^21p^6$, and the others to a closed-shell core with an outer layer (the mantle of the nucleus) containing the number of spherons (helions[6], He^4, tritons, H^3, or dineutrons) required to surround the core in closest packing.

Triangular (icosahedral) closest packing, as found, for example, in the intermetallic compound[7] $Mg_{32}(Al,Zn)_{49}$, involves the sequence 1, 12, 32, 72 of spheres in successive layers. These numbers are approximated by the equation $n_0=(n_i^{1/3}+1{\cdot}30)^3-n_i$, in which n_0 is the number of spheres in an outer layer and n_i is the number in the core. (The form of this equation corresponds to assigning equal effective volumes to the spheres, and the value of the constant reflects the nubbling of the surface and the packing of outer spheres into pockets of the core.) This equation can be applied to obtain the number of spherons in the successive layers in a nucleus, and thus to obtain the sequence of nucleonic energy-levels. Sub-shells (with given value of l) occurring once (as $1s$, $1p$, etc.) are assigned to the mantle of spherons, those occurring twice ($1s$ and $2s$, for example) to the mantle and next inner layer, and so on. Thus I interpret the configuration for N or $Z=50$, given above, as representing 8 neutrons or protons in the core $(1s^21p^6)$ and 42 in the outer layer $(2s^22p^61d^{10}1f^{14}(1g9/2)^{10})$.

The application of the packing equation leads to a sequence of levels essentially as given by Mayer and Jensen, but often with sub-sub-shells for different layers being filled over overlapping ranges of values of N or Z. For example, the $3s1/2$, $2d3/2$, and $1h11/2$ sub-sub-shells all begin to be occupied at about N or $Z=60$ and are all completed at about N or $Z=82$.

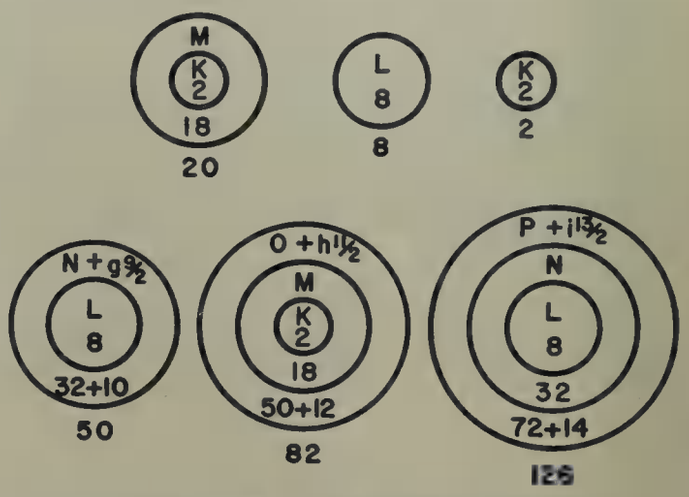

Fig. 1. The magic-number structures of atomic nuclei

The configurations found in this way for the magic numbers are given in Table 1 and Fig. 1. Each of the first two represents a completed shell. The third (20) has a completed shell as core and another as mantle. Each of the others has a core of a completed shell or two completed shells, with a mantle that is required by the packing to include a sub-sub-shell $(1g9/2)^{10}$ for 50, $(1h11/2)^{12}$ for 82, and $(1i13/2)^{14}$ for 126. Until 184 is reached, there are no other values of N or Z for which the packing equation leads to a core consisting of layers that are completed shells.

Table 1. Nucleon Configurations for Magic Numbers

N or Z	Mantle	Core or outer core	Inner core
2	$1s^2$		
8	$1s^21p^6$		
20	$2s^21p^61d^{10}$	$1s^2$	
50	$2s^22p^61d^{10}1f^{14}(1g9/2)^{10}$	$1s^21p^6$	
82	$3s^22p^62d^{10}1f^{14}1g^{18}(1h11/2)^{12}$	$2s^21p^61d^{10}$	$1s^2$
126	$3s^23p^62d^{10}2f^{14}1g^{18}1h^{22}(1i13/2)^{14}$	$2s^22p^61d^{10}1f^{14}$	$1s^21p^6$

I conclude that the stability that characterizes the magic numbers results from the completion of shells for a single layer (2, 8) or two layers (20) of spherons, or, for the larger magic numbers (50, 82, 126), for the core layers, the mantle having a completed shell plus a completed sub-sub-shell.

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Size Analysis in the Sub-sieve Range by Electronic Counter

THE electronic apparatus for the rapid measurement of particle content and size distribution, known as the Coulter counter[1], is finding increasing application in many fields. A known volume of an extremely dilute suspension of particles in a conducting fluid flows through a standard interchangeable aperture having an immersed electrode on either side. The particles traverse the aperture substantially one at a time, causing an attendant change in electrical resistivity proportional to the volume of the particle passing through the aperture. The amplified signals pass through a gate circuit which ensures that only pulses greater than a pre-selected threshold value are counted; thus, the pulse count records the number of particles greater than a certain size. A typical count requires about 5–25 sec and is performed at a rate up to 600 particles/sec. A size range of about 500–0·5μ can be covered, therefore overlapping with sieving, microscope and sedimentation methods.

After applying certain corrections (for example, for the effect of coincident particle passage) and calibration constants, the basic data relate the numbers of particles greater than a stated size to the particle volume. This information is readily converted into the relationship between cumulative volume above a stated size (V) and particle size (X).

Size analysis results are usually quoted as cumulative volume (or weight) percentage above (or below) a stated size versus size. For this the total volume, V_∞, or weight of particles in the measured sample must be known. However, the instrument response to sub-micron particles is obscured, if not by insensitivity then by factors such as background noise and insufficient counter frequency response. Thus, the finest material in a distribution may be below the range of measurement, so that the cumulative data cannot be expressed as a fraction or percentage.

It would be possible to obtain the total solid volume gravimetrically, but one of the potential advantages of the Coulter counter is that, in the suspension prepared for analysis, the concentration of solids need not be known; additionally, the gravimetric analysis would need to be highly sensitive. The maker's instruction manual suggests a trial and error method in which the total volume of particles traversing the aperture is estimated by choosing the value providing the best straight line when cumulative percentage is plotted against size on a probability X logarithmic grid. A similar curve-fitting procedure was suggested by Batch[2]. Simple extrapolation of the plot of cumulative volume against size either on simple arithmetic or logarithmic X arithmetic co-ordinates affords another approximate method of estimation. Experience indicates that both methods are unreliable.

The problem is determination of an unknown asymptote[3], and a somewhat analogous situation exists with the sedimentation balance where an inordinately long time is necessary for the finest material to sediment, or because it remains permanently in suspension on account of Brownian movement[4]. A direct method for graphically estimating the missing finest material[5], or what amounts to the same thing, the value of V_∞, is presented here. While sub-micron material is the main concern here, the method may be applied to any size below the range of the aperture in use.

The equations of size distribution[6] assumed are the Gates–Gaudin–Schuhmann type:

$$V = V_\infty\left[1 - \left(\frac{X}{X_0}\right)^N\right] \qquad (1)$$

and the Rosin–Rammler type:

$$V = V_\infty e^{-(X/X_0)^n} \qquad (2)$$

where X_0 is a parameter of mean size, and n and N parameters of distribution spread. These are closely similar for the finest 20 per cent of the material and their correspondence at 30 per cent is within 1 per cent absolute. Consequently one or other equation could be used when the missing material is of the order of 20 per cent of the total volume counted. Correspondence with the logistic and probability distributions is less good, being about 0·5 per cent in absolute error over the finest 10–15 per cent of the material.

Equation (1) can be represented by the pair of equations[4]:

$$X_{r-1} = qX_r \qquad (3)$$

and:

$$\left(\frac{V_{r-1}}{V_\infty} - 1\right) = q^N\left(\frac{V_r}{V_\infty} - 1\right) \qquad (4)$$

while equation (2) can be represented by the pair of equations[4]:

$$\frac{V_{r-1}}{V_\infty} = \left(\frac{V_r}{V_\infty}\right)^{q^n} \qquad (5)$$

together with equation (3), where r is an integer increasing as X decreases and as V increases and $q > 1$ and is constant. A solution of equations (4) and (5) is $V_{r-1} = V_r = V_\infty$; by plotting V_{r-1} against V_r on a squared grid for equation (4) and on a log–log grid for equation (5), V_∞ is obtained from the point of intersection of the resultant line with the line, $V_{r-1} = V_r$.

Table 1. SIZE ANALYSIS OF −300 B.S.-MESH QUARTZ BY COULTER COUNTER

Calculated values (from counter data)		Interpolated values ($q=\sqrt{2}$)	
Particle size (X, microns)	Cumulative vol. (V, arbitrary units)	Particle size (X, microns)	Cumulative vol. (V, arbitrary units)
60·40	0	33·12	5,100
38·68	3,528	23·44	9,100
24·19	8,792	16·56	12,700
15·28	13,523	11·72	15,600
10·68	16,365	8·28	17,600
9·33	17,183	5·86	18,669
7·40	18,103		
5·86	18,669		

The procedure for applying this method is to plot the original data V against X and interpolate values of V_{r-1}, V_r, V_{r+1}, etc., corresponding to values of X_{r-1}, X_r, X_{r+1}, etc., diminishing in geometric progression in accordance with equation (3) (see Table 1). The last actual reading of V and X should be included in this sequence. Interpolation at this stage has the advantage of smoothing experimental errors. Having determined V_∞ from a plot of V_{r-1} against V_r (Fig. 1) the size distribution Y against X ($Y = V/V_\infty$) is obtained in the usual manner.

For this particular example, V_∞ has been estimated to lie in the range 20,500–20,600 by independent observers. The ordinary extrapolation method, on the other hand, yielded estimates ranging from 19,500 to 21,200. The estimation of V_∞ from the plot of V_{r-1} versus V_r can be

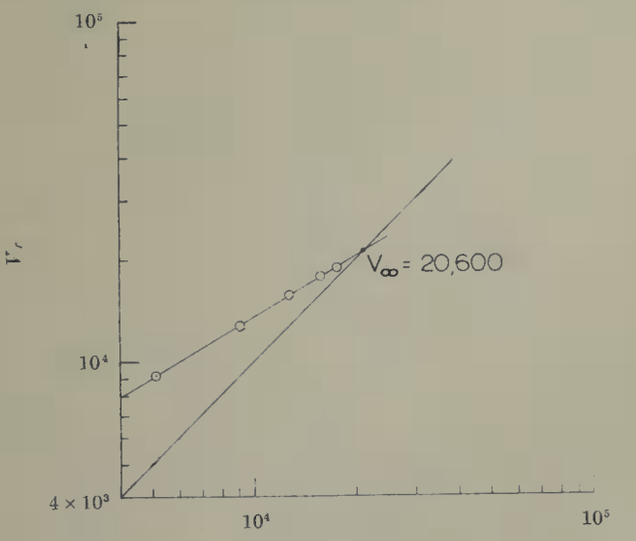

Fig. 1. Graphical determination of V_∞. Data from Table 1. $q=\sqrt{2}$; last value of $X = 5{\cdot}86\ \mu$; $V = 18{,}669$

made as accurate as one desires. Larger graphs can be drawn and more points taken, either by diminishing the ratio, q, or by taking overlapping sets of points; for example, for a ratio of $\sqrt{2}$, take the sets 5·86, 8·28, . . ., and 6·0, 8·5, . . . and 6·5, 9·2, . . . etc.

If $n = 1$ the values of X may be selected in arithmetic progression; this case has been considered previously[4]. Should the plotted points not lie on a straight line the curve would probably fit another function having an unknown asymptote. However, the last point should be sufficiently close to the line $V_{r-1} = V_r$ for an extrapolated curve to give a reliable estimate of V_∞.

This graphical procedure has been used in a number of similar applications all involving unknown asymptotes[4,5,7,9]. Finally, a warning must be issued: this is an extrapolation technique; there is no certainty that the size distribution law outside the measured range is the same as that inside. Thus, results obtained from this method must be used with discretion; it is not a substitute for an effective and rapid particle size analysis technique for the submicron region, the search for which must continue.

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Ionization Times in Shock-heated Potassium Vapour

The sudden heating of a gas in a shock wave has often been used to study relaxation effects[1–3]. Most experiments on relaxation times for ionization have been carried out with inert gases, which have first ionization potentials in the range 12–24 V. These measurements have suggested[1,4,5] that the atoms are ionized via excited states; but the results may have been influenced by the unknown effect of impurities.

The monatomic character of inert gases, and the consequent absence of complications due to dissociation, make the inert gases attractive at first sight; but in fact the high ionization potential allows only a small amount of ionization in a pressure-driven shock-tube and almost any impurity may dominate the ionization process. Moreover, the high temperature required to achieve a reasonable degree of ionization results in a short cooling time, which may be less than the ionization time if the density is low; and complications arise because of secondary ionization and ion excitation, since the relevant critical potentials are of the same order.

It has often been pointed out[6] that these difficulties could be avoided by using alkali metals (these have ionization potentials of 4 or 5 V, whereas the first excitation potential of their ions is several times this figure). But the great technical problems of passing shocks through such vapours have to be overcome. Such a shock tube is now in operation and ionization relaxation times have been determined by measuring the current in probes flush with the sides of the tube.

The shock tube, which was specially developed for alkali metal vapours, is of stainless steel and has ground-in stainless steel valves and a copper diaphragm. It is immersed in an oil vapour furnace at 300°–400° C. Liquid potassium metal is fed into a boiler. The latter can be isolated from the tube, cooled during evacuation, and then opened and allowed to heat up to admit the vapour after the shock-tube has been isolated from the pumps. The boiler is kept at a temperature some 10° C lower than that of the furnace. Initial potassium pressures of 0·3–3 torr can be obtained by varying the temperatures of the boiler and furnace. Helium–argon mixtures at a pressure of about 15 atmospheres are used in the compression chamber to produce shocks with Mach numbers of between 3 and 8 in the potassium vapour.

Metal probes of 'Nilo K' (an alloy of iron, cobalt and nickel), about 1 mm diameter and insulated from the shock-tube by a ceramic seal, are fitted flush to the wall of the expansion chamber and connected through a series resistor to the positive terminal of a 4·5-V battery, the other terminal being connected to the metal shock tube. The series resistor is adjusted by experiment to have about the same value as the resistance of the probe in the equilibrium plasma behind the shock wave. Oscillograms of the probe voltage indicate the electron current to the probe. Probes mounted at two stations 13 cm apart show traces on a double beam oscilloscope from which the shock speed can be measured. The first probe also operates a trigger-amplifier which triggers this oscilloscope immediately and also triggers a fast time-base oscilloscope after a variable delay. This delay is arranged by experiment so that the fast oscilloscope is triggered just before the shock reaches the second station. The fast oscilloscope records on the upper beam the probe voltage at the second station and on the lower beam the voltage on another probe mounted opposite this with a small reflecting obstacle immediately behind it. Fig. 1 shows a typical trace. The lower trace shows a faster rate of ionization, but no change in the onset time. This indicates that there is no dormant period between the shock and the initial fall of the probe voltage.

Relaxation times τ (defined as indicated in Fig. 1) have been measured for shock Mach numbers M in the range 3·2–4·6 and initial particle densities n from 6×10^{15} to 2×10^{16} per c.c. Higher densities and shock Mach numbers resulted in relaxation times less than 1 μsec which could not be resolved by the probe. Lower densities and Mach numbers failed to trigger the amplifier, probably because of a reduction in running time.

Fig. 2 shows a graph of log $n\tau$ against $1/T$ (where T is the temperature of the shocked gas calculated from M). If the ionization relaxation time obeys an Arrhenius law of the type :

$$\tau \propto (1/n) \exp (eV/kT) \qquad (1)$$

where V is some critical potential, the slope of the graph should give V. A least squares fit gives $V = 1{\cdot}9 \pm 0{\cdot}3$ V. The first potassium excitation potential is 1·6 V. Since the ionization potential of potassium is 4·3 V, these results indicate a multi-stage ionization process dominated by the rate of excitation of the atom. The measured values of τ are for values of n and T much lower than those used by previous investigators. If the measurements for

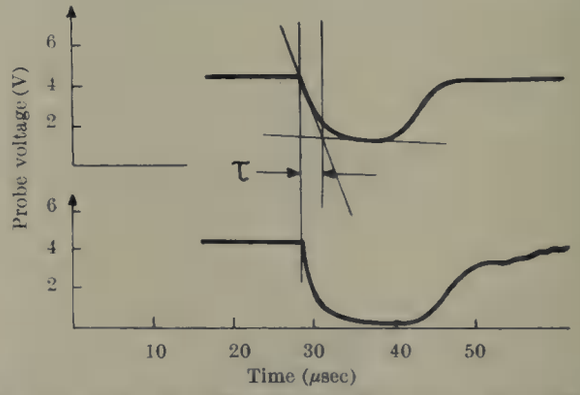

Fig. 1. Typical probe voltage traces. Upper trace, plain probe; lower trace, reflector probe

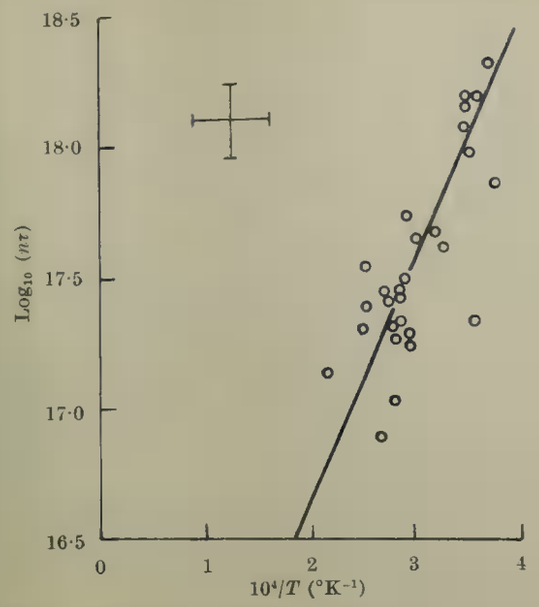

Fig. 2. Ionization times in shock-heated potassium vapour

argon[1,7] are extrapolated according to expression (1) to our values of n and T, values of τ some 10^9 times greater than for potassium are obtained. This is reasonable considering the much lower potentials and higher cross-sections of the alkali metals. Though experiments have previously been performed on argon seeded with small amounts of alkali metal[8] these experiments are, so far as we know, the first of this type to be made in a pure metallic vapour.

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METALLURGY

Interconnexion of Silicon in Modified Aluminium–Silicon Eutectic

When the aluminium–silicon eutectic is rapidly frozen, or when sodium is added in small quantities (< 0·017 wt. per cent), the structure apparently is modified from an acicular form to a globular form of the silicon phase[1–7]. The reason for the modification is still a matter of controversy[8], and a review of present-day theories is given by Chadwick[1].

All the theories presented so far claim that the fine dispersion of silicon particles is produced by an increased nucleation frequency of silicon particles at a temperature lower than the equilibrium eutectic temperature. The lowering of the temperature is attributed to either a reduction in the initial nucleation frequency[1,3,6,9–12] or restricted growth[2,4,7,13–15].

All present theories assume that each of the silicon particles in the modified structure is separate from, and not connected to, a neighbouring particle. The work reported here shows that this assumption is not valid.

A rapidly cooled sample of high-purity aluminium–silicon eutectic was polished metallographically to reveal the modified structure. The sample was then etched for 24 h in a 10 per cent hydrochloric acid solution, and for 1 h in a solution of 2·5 c.c. hydrochloric acid, 2·0 c.c. nitric acid, 0·75 c.c. 40 per cent hydrofluoric acid, and 40 c.c. water to dissolve the aluminium phase and leave the silicon untouched.

The silicon phase in this modified structure was found to be interconnected in any one grain, even when the grains were 2 mm in diameter. The same result was obtained when the structure of the sodium-modified alloy was examined.

From these observations, it is apparent that in the aluminium–silicon eutectic the silicon phase grows from a common nucleus in each eutectic grain in both the rapidly cooled and sodium-modified structures. In fact, the structure has not been 'modified' in the normal usage of this word. There is no change in the basic growth mechanism, and the structure is not globular. The silicon takes up a very fine interconnected acicular structure.

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GEOLOGY

Re-classification of the Ethiopian Cainozoic Volcanic Succession

Recent exploration and field-work in Ethiopia, in particular gravity–geology surveys made by Prof. P. Gouin, Dr. A. S. Rogers and me, have shown the Cainozoic volcanic succession in association with the Ethiopian Rift System to be more complicated than previously suspected.

The classic work of Blanford[1] established the existence of an older, extensive, pre-rift series of lavas, the Trap Series, covered by a younger, post-rift series of lavas, the Aden Series, the latter largely confined to the rift-system itself. Furthermore, Blanford divided the Trap Series into an earlier group of basalts, the Ashanghi Group, unconformably overlain by basaltic and silicic lavas and tuffs, the Magdala Group. This classification was generally accepted until the work of Merla and Minucci[2], who denied the existence of Blanford's unconformity, considering the tilting of the Ashanghi Group basalts to be due to post-Trappean tectonic movements. Merla and Minucci's revision of the nature of the Trap Series succession has been largely accepted by later workers, including Dainelli[3] and myself[4], though Blanford's terminology has been retained on the basis of lithology.

However, the recent discoveries of unconformities within the Trap Series in various parts of Ethiopia, by Jepsen and Athearn[5], Rogers[6] and me[7], have led Rogers and me to a reconsideration of Merla and Minucci's arguments and a re-examination of the critical Ashanghi–Magdala region. The results of this new work (details to be published elsewhere[8]) have included the re-establishment of Blanford's unconformity, and the implications of this finding together with additional data from recent studies in the Ethiopian Rift System are summarized here.

The Trap Series, as defined by Dainelli[9], can now be divided into three major units: (3) great shield volcanoes of the Plateaux, composed of basalts, alkaline silicics and undersaturated lavas. Generally conformable on: (2) fissure and central-type basalts, with silicic lavas and pyroclasts becoming more abundant near the top. Unconformable on: (1) fissure basalts, with localized thick silicic lavas in Afar. Unit 1 is the Ashanghi Group of Blanford, and units 2 and 3 the Magdala Group. It would, however, be preferable to exclude unit 3 from the Magdala Group for reasons of age-range, localization and petrology. The term 'Shield Group' might be acceptable, and will be used here.

The re-establishment of Blanford's unconformity has led to the important finding that the Ashanghi Group lavas were down-warped (and flowed down ?) into Afar and the Main Ethiopian Rift during and immediately subsequent to their extrusion[4,8]. The sites of the fissure-feeders for these lavas coincided with the Plateau–Afar structural margin[4,7], where down-warping was particularly severe and complicated by strong north–south compressional movements. There is evidence at Amba Alaji (12·58° N., 39·32° E.) that these compressional movements had begun during the deposition of the now isoclinally folded Jurassic marine sediments[8,10]. Furthermore, the Ashanghi lavas seem to have been largely confined to the region and margins of the down-warped proto-rift system; they have been dated as Eocene by Lipparini[11] and Grasty *et al.*[12].

The ensuing initiation of the Arabo–Ethiopian Swell in the Upper Eocene resulted in a moderate uplift of the whole region[8,13] (cf. Dainelli[2], who considered the larger part of the uplift of this Swell to have occurred at this time). This uplift was followed, over most of Ethiopia, by a duration of volcanic quiescence sufficient for appreciable peneplanation to occur across the warped Ashanghi lavas along the Plateau–Afar margin, prior to the first Magdala Group eruptions. On the northern Somalian Plateau, however, there was either little or no break between the Ashanghi and Magdala eruptions, or else a very small degree of uplift in the Upper Eocene.

Unlike the Ashanghi lavas the Magdala lavas are thickly developed over much of the Plateaux away from the Rift System, perhaps because of their dominant extrusion from aligned centres on a gently sloping surface. These centres lay just outside the outer structural margin of Afar[4,7] on a surface suffering contemporaneous slight upwarping towards the Rift System. Important fissure eruptions of basaltic and silicic lavas occurred along the inner structural margins of Afar, lavas which were included by Gortani and Bianchi[14] as the earliest lavas of the Aden Series. The Magdala Group lavas have been dated as Oligocene and early Miocene[3,12].

In the Burgdigalian–Helvetian period there occurred the major uplift of the Arabo–Ethiopian Swell together with rift faulting on a large scale[13,15]. The great shield volcanoes, of which the culminating example is that of Semien[16], were initiated at, or shortly after, this tectonic paroxysm. Despite the tectonic intervention, however, the lack of significant unconformity between the Magdala Group and the Shield Group indicates a continuing though modified single volcanic episode. The Shield Group volcanoes all lie outside and in many cases well away from the Rift System. Until more radiometric ages are available it remains uncertain what form, if any, volcanicity took within the Rift System during Shield Group times, but it seems likely that the upper part of the Magdala lavas of the Rift System are temporally (but not petrologically) equivalent to the Shield Group of the Plateaux. The Shield Group is dated as late Miocene–early Pliocene, but isolated volcanoes continued their activity throughout the Pliocene and even into the Pleistocene. A good example of such late activity is provided by the alkaline rhyolitic centre of Mt. Kakka on the Somalian Plateau.

As previously indicated by me[4], there was no obvious interval between the last Trap Series eruptions and the first Aden Series eruptions; indeed, recent work now suggests an overlap and a corresponding difficulty in drawing a precise line between the two, especially within the Rift System. The earliest lavas ascribed to the Aden Series are the Pliocene hyperalkaline, coarsely crystalline lavas of which the famous Adua–Axum suite on the northern Ethiopian Plateau is typical. These lavas are aligned along approximately east–west lines.

Pleistocene volcanicity occurred throughout at least two phases of renewed uplift of the Arabo–Ethiopian Swell[13], being characterized by violent silicic eruptions in the Rift System but by basalts on the Ethiopian and Somalian Plateaux. Such basalts dammed back Lake Tana in the down-warped and tilted Tana basin. In late Pleistocene and Holocene times transcurrent faulting along the Main Ethiopian Rift[7,17] was accompanied by carbonatitic activity, while the latest lavas erupted from the fissures and volcanoes of the Rift System have been either scoriaceous olivine basalts or pantelleritic and comenditic obsidians.

No detailed table of the succession of lavas and tectonic events during the Cainozoic in Ethiopia is presented here, as information on the Trap Series succession on the southern part of the Ethiopian Plateau is still being gathered. More important, as hinted in this communication, local variations and complexities both in lava thickness and types and in contemporaneous tectonic movements are proving to be more intricate than can be satisfactorily expressed in any correlation table at present. In this regard the recent work of Merla[13] on erosion surfaces in south Ethiopia and north Kenya, and in particular his consideration that the Plateau surface is the result of post-Trappean peneplanation during the Oligocene, must be treated cautiously before being extended into central Ethiopia[8]. Indeed, the conclusions of different investigations of the behaviour of erosion surfaces in southern Ethiopia are so contradictory[13,18,19] as to emphasize the need for a dispassionate résumé to indicate the critical regions needed for further examination.

Finally, while this article has been briefly concerned with the major Cainozoic volcanic episode in Ethiopia, it must be noted that there was important vulcanicity during Mesozoic times along the present Red Sea coastal region. Both north of Massawa and east of the Danakil Alps andesitic lavas occur which are unconformably overlain by the thick Eocene–Miocene Dogali geosynclinal sediments of the proto-Red Sea trough; in the Danakil Alps these same lavas rest unconformably on late Jurassic limestones, thus suggesting an end-Jurassic or early Cretaceous phase of vulcanism.

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Palaeomagnetism of the Isachsen Diabasic Rocks

In a previous article by two of us[1] it was suggested that a belt of diabasic rocks south of Isachsen in the Canadian Arctic Archipelago appeared to be of Cretaceous age on the basis of its palaeomagnetism. On the other hand, whole rock potassium/argon age determinations on two of the samples studied (1–*A* and 10–*B*) indicated a Permian age (241 and 249 m.y.), in conflict with the palaeomagnetic data. No reasonable explanation for this incompatibility could be advanced at the time and, since then, one of us (R. K. W.) has re-determined the age of one of the samples (10–*B*), using a mass spectrometer with higher sensitivity than was used in the original determinations. Another sample (9–*B*) was sent to the Isotope Branch laboratories, U.S. Geological Survey, and a third one (9–*A*) was split and one half of it was sent to Dr. N. J. Snelling at the University of Oxford, while the other half was dated in the laboratories of the Geological Survey of Canada with the new high-sensitivity instrument. The ages obtained in these four whole rock determinations are listed in Table 1.

Table 1

Sample	Laboratory	% K	% ^{40}Ar (radiogenic)	^{40}Ar/^{40}K	Age* (m.y.)
10–*B*	Geological Survey of Canada	0·93	39	0·00656	109
9–*A*	,, ,,	0·96	40	0·00660	110
9–*A*	Oxford (ref. 2)	0·94	39	0·00618	103
9–*B*	U.S. Geological Survey (ref. 3)	0·75	66	0·00612	102

* Constants: $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ y^{-1}; $\lambda_e = 0{\cdot}585 \times 10^{-10}$ y^{-1}; ^{40}K = 0·0119 atomic per cent.

It is apparent from the consistency of these determinations that the rocks dealt with in the original paper[1] are of Lower Cretaceous age and that the pole position derived from their mean palaeomagnetic direction should be considered as a fairly reliable one for Lower Cretaceous time with respect to the North American Continent.

We thank Dr. N. J. Snelling of the University of Oxford and Dr. S. S. Goldich, formerly of the U.S. Geological Survey, for their assistance.

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MINERALOGY

Interstratified Mineral of Illite and Montmorillonite

A yellowish-grey tuff, about 1 m thick, is distributed over a considerable area of the Sorachi coal field, Hokkaido, Japan[1], as a member of the Noborikawa coal-bearing formation. The tuff contains a small amount of quartz and plant fragments, but is generally homogeneous.

Table 1. X-ray Powder Data of 29 Å Interstratified Mineral (Illite–Montmorillonite)

Air dried *d*(Å)	150° C	300° C	450° C	600° C	750° C	E.G.	G.	NH_4NO_3
29·4	29·4	29·4						
11·9	11·11	10·3	9·94	10·3	10·5	11·9	12·8	11·1
						9·51	9·51	
						7·14		
5·07	5·04	5·01	4·95	5·04	5·10	5·13	5·90	5·13
								5·01
4·51	4·51		4·48	4·51	4·51	4·46	4·46	4·48
					3·36	3·34	3·34	3·36
					(m)	(m)	(m)	
3·28	3·30	3·35	3·30	3·34				3·28

Values represent the maximum peak positions of reflexions.
(m) means that it may be multiple reflexions of quartz and interstratified mineral.
E.G., treated with ethylene glycol. G., treated with glycerol.

Having dispersed some of the material in water, the minus-two-micron fractions of the tuff were separated by the sedimentation method; Fig. 1 shows its electron micrograph. The X-ray powder patterns and data on the well-oriented specimens are shown in Fig. 2 and Table 1. The specimens were dried in air, heated at 150° C, 300° C, 450° C, 600° C,

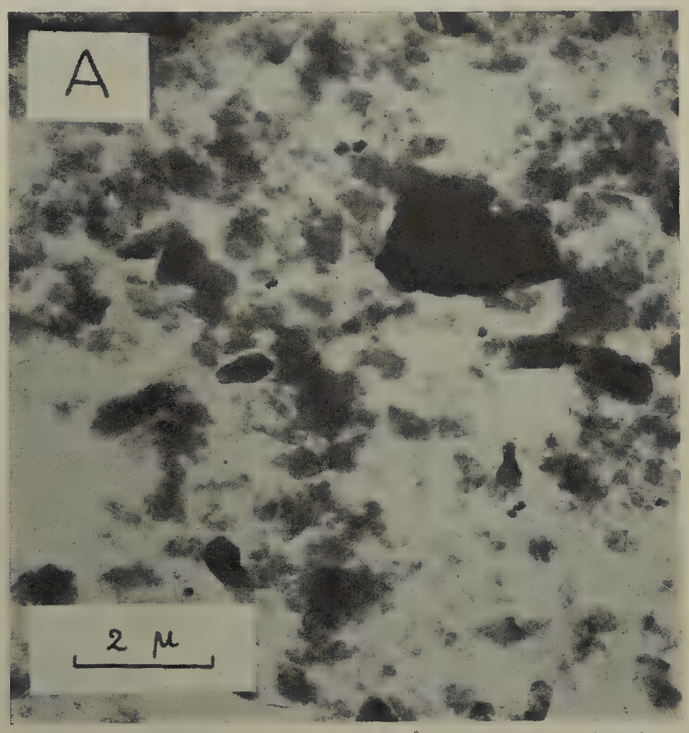

Fig. 1. Electron micrograph of 29 Å interstratified mineral

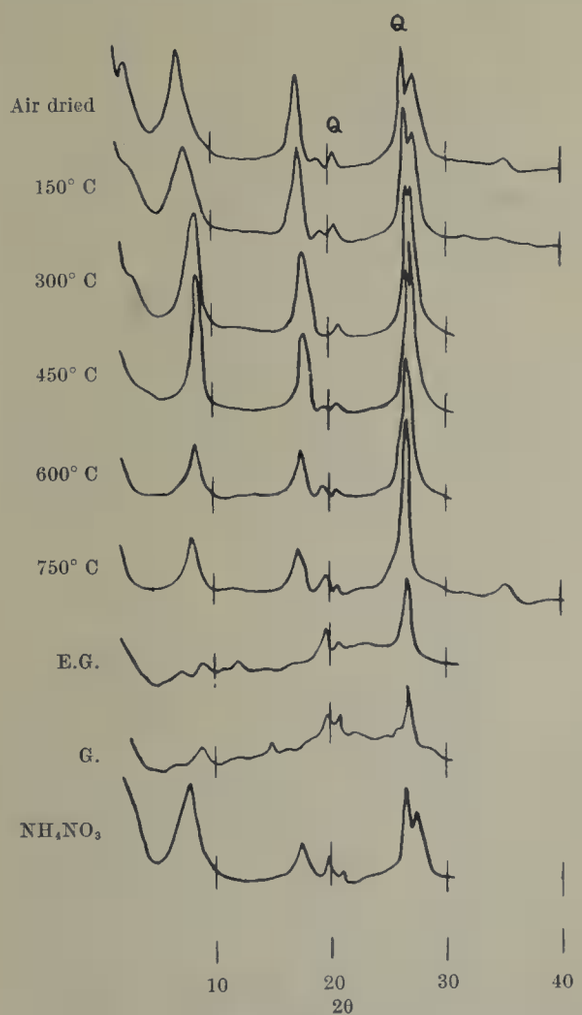

Fig. 2. X-ray powder patterns of the interstratified mineral. Q, reflexions due to quartz; E.G., treated with ethylene glycol; G., treated with glycerol. Operation conditions: Ni filtered copper radiation. 35 kV, 15 m.amp. Scanning speed, 1°/min; scale factor, 16; multiplier, 1; time constant, 4; receiving slit, 0·4 mm

Table 2. Chemical Composition of the Interstratified Mineral

	%		%
SiO_2	52·64	MgO	2·22
TiO_2	0·38	Na_2O	0·24
Al_2O_3	27·77	K_2O	3·72
Fe_2O_3	0·89	H_2O (110° C)	3·04
FeO	0·98	ig loss	6·50
MnO	0·02	Total	99·58
CaO	1·28		

Table 3. Observed and Calculated Spacings

Air dried		450° C	
$d_{obs.}$	$d_{cal.}$	$d_{obs.}$	$d_{cal.}$
29·4 Å	28·8 Å		
11·95	12·02	9·94 Å	9·94 Å
5·07	5·08	4·95	4·965
3·25	3·247	3·30	3·309

The calculation is based on the model shown in Fig. 4.

750° C, for 1 h, and treated with ethylene glycol, glycerol and ammonium nitrate. The chemical compositions and differential thermal analysis curves of both natural and piperidine-treated specimens are shown in Table 2 and Fig. 3.

All these data correspond to those of the so-called random interstratified minerals of illite and montmorillonite reported by many investigators, but the X-ray reflexion at about 29 Å in the natural specimen (uppermost in Fig. 2) cannot be expected from the random structure. Sato has proposed[3] that the interstratified mineral would take its layer arrangements to minimize the total interaction energy, and that it should be treated for at least Rechweite

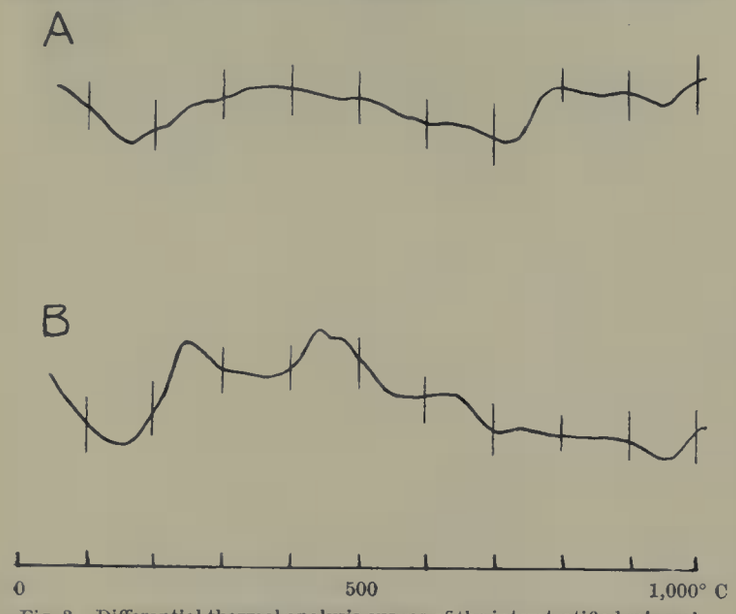

Fig. 3. Differential thermal analysis curves of the interstratified mineral. *A*, Natural; *B*, treated with piperidine

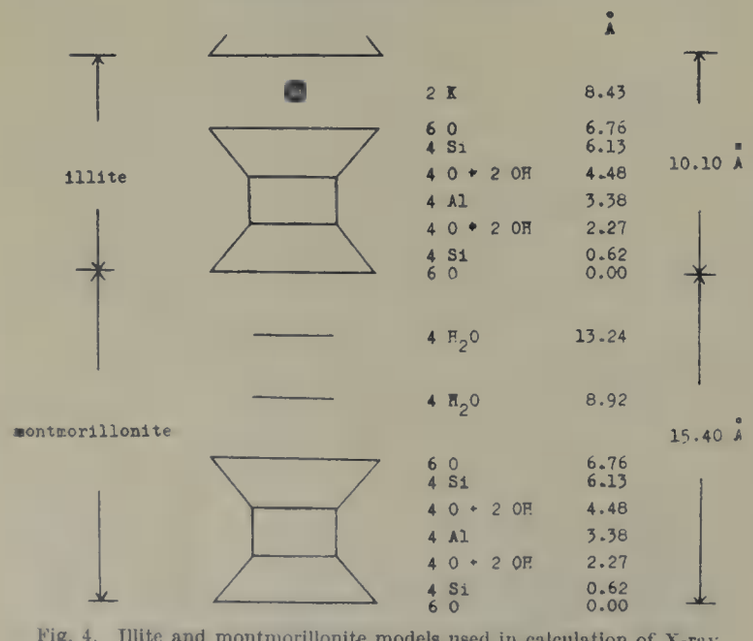

Fig. 4. Illite and montmorillonite models used in calculation of X-ray intensity

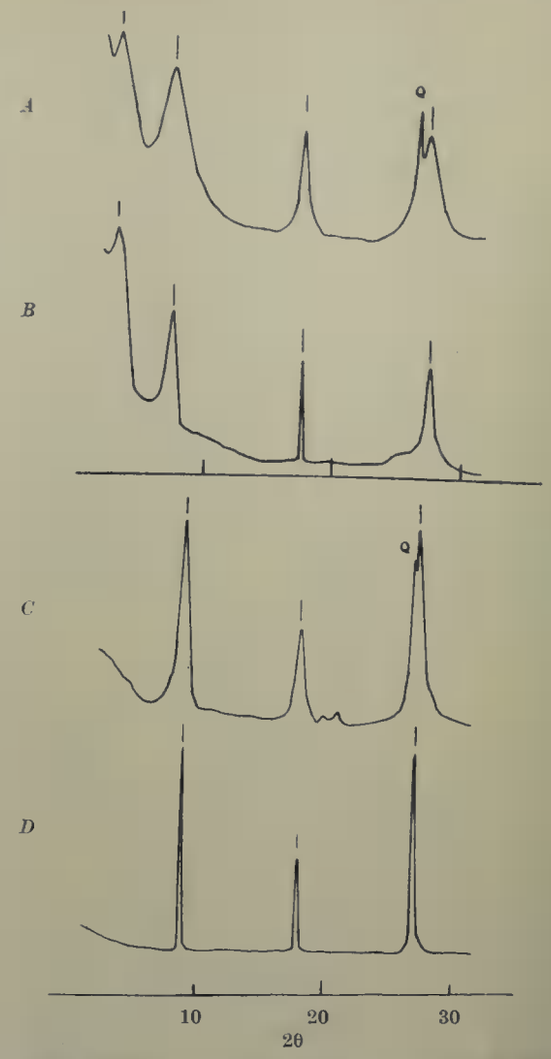

Fig. 5. X-ray reflexion curves along (00ζ). *A* and *B*, air dried, observation, calculation, $N=60$ (total number of layers); *C* and *D*, heated at 450° C, observation, calculation, $N=150$

$g=1$, not $g=0$ (random). With this in mind, X-ray reflexion curves along (00ζ) in the air-dried specimen and the specimen heated at 450° C—the intensity of which was calibrated at low angles in consideration of the slit system used—were carefully compared with mathematical curves. The calculation was based on the models shown in Fig. 4. As a consequence, it was found that the specimens were an interstratified mineral of illite and montmorillonite, in which the component of illite (W_I) is 0·72, that of montmorillonite (W_M) is 0·28, the probability of finding illite succeeding illite (P_{II}) is 0·611, and the probability of finding montmorillonite succeeding montmorillonite (P_{MM}) is 0·0. The result is shown in Fig. 5 and Table 3 (and see graph in ref. 2).

We thank Prof. T. Sudo for his advice.

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CRYSTALLOGRAPHY

Pairs of Spiral Etch Pits in Ice Crystals

In an earlier communication[1], I reported observations of spiral etch pits in ice crystals which had quite large step heights (200–2000 Å). At that time no detailed explanation of their origin was offered; but in the course of further investigations[2] of ice crystal etch pit, these etch pits were postulated to correspond to dislocations. In

many crystals, however, the step height of the spiral etch pits is quite large in comparison with the Burgers vector, and it is thus impossible to determine the causal factor[3].

Recently, an attempt was made to verify the real mechanism involved in etching in ice crystals, and it was found that pairs of etch pits may develop on the {0001} surface in which the pairs consist of one right- and one left-handed spiral. These are often remarkably similar to those one would expect from a Frank–Read source (Figs. 1 and 2). Sometimes, pairs of pits with a unidirectional spiral are observed (Fig. 3). It is of interest to note that each pair of etch pits is closely aligned to crystallographic orientations such as $<10\bar{1}0>$ or $<11\bar{2}0>$. In Figs. 1 and 3, the distance between a pair of pits is about 8μ and in Fig. 2 the distance is 50μ.

If we assume that these spiral etch pits correspond to pure screw dislocations lying on a prism plane, the Burgers vector should be $<0001>$, which vector, although possible, has never been reported in ice crystals or in any other hexagonal crystals. If this assumption is correct, the existence of paired etch pits consisting of one left- and one right-handed spiral implies a dislocation half loop on a prism plane (Figs. 1 and 2). With regard to the large angle of the pitch of the spiral, one interpretation, based on the presence of a dislocation or a helical dislocation, has been given by Ellis[4] and Amelinckx *et al.*[5], and another by Lang[6] which is not based on the presence of dislocations. It is possible that the large step heights observed are not formed from screw dislocations with Burgers vectors of this magnitude, but develop during the etching process from aggregations of asymmetrical spirals which develop about the point on the surface at which

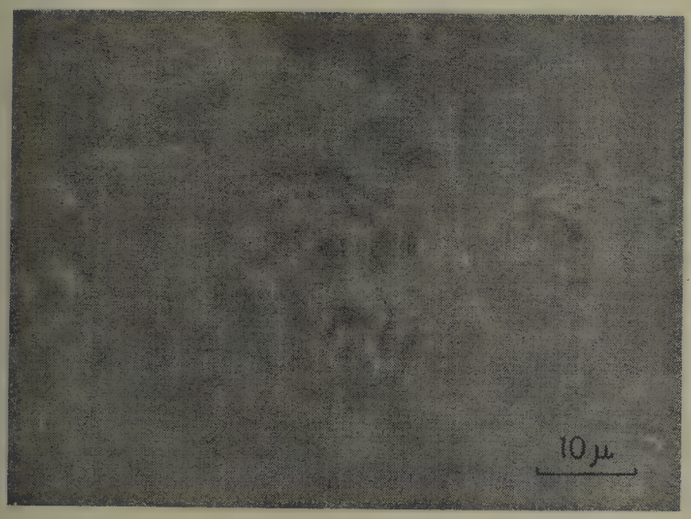

Fig. 1. A pair of etch pits on a {0001} plane. Note that one is right-handed and the other is left-handed

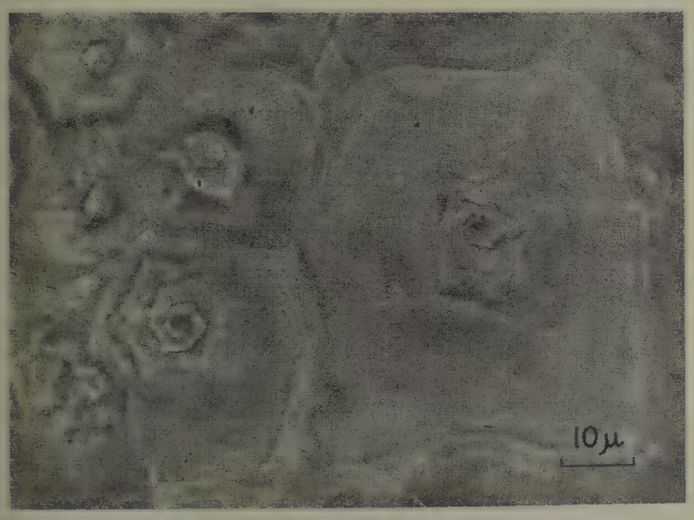

Fig. 2. A pair of fairly widely separated etch pits on a {0001} plane. One spiral is right- and the other left-handed

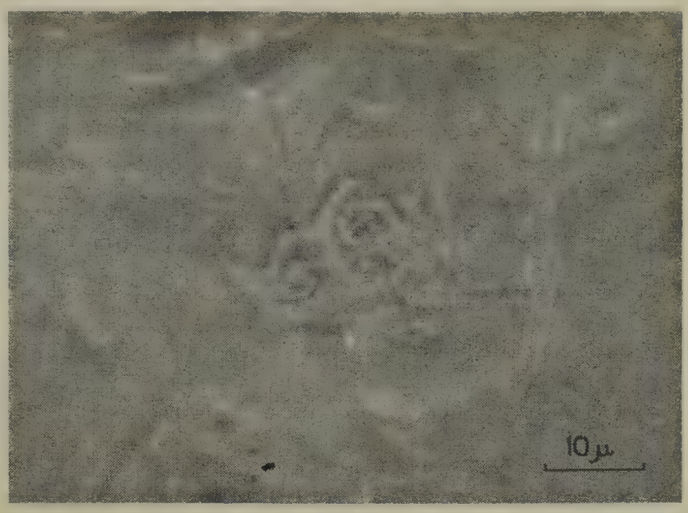

Fig. 3. A pair of etch pits: with unidirectional spirals

the dislocation emerges. This closely resembles the mechanism proposed by Amelinckx *et al.*[5] which requires a helical dislocation; but there is no reason why a screw dislocation could not develop such a spiral. Although this appears to be a plausible explanation of the large angle of pitch observed, further detailed investigation is required for any definite conclusions.

Recently, the first direct observation of dislocation in ice crystals has been reported by Hayes and Webb[7], using Lang's X-ray transmission method. They found that dislocation segments in the (0001) plane are quite long and are oriented parallel to $<10\bar{1}0>$ and $<11\bar{2}0>$. Judging from the results of direct observations and from successive etching of basal surface, which give a three-dimensional picture of the dislocation arrays, the non-basal segments of these dislocations are probably very short, and one may expect that there would be a long distance between a pair of pits.

If this speculation is correct, dislocations with $<0001>$ Burgers vectors may exist in ice crystals and may provide a clue to an understanding of some unexplained observations of dislocation movement in ice crystals.

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CHEMISTRY

Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces: Evidence for Catalytic Activity of *F*-centres

We have recently found that diphenyl picrylhydrazyl undergoes intermolecular oxidation and reduction quite rapidly on carbon black (Cabot: 'Carbolac 2') and on a high area TiO_2 (anatase) which has almost a monolayer of sulphuric acid on its surface.

With 2 g of diphenyl picrylhydrazyl dissolved in 250 c.c. of benzene and 25 g of anatase (263 sq. m/g, containing 7·55 wt. per cent of sulphuric acid) the reaction is more than 90 per cent complete in 90 min.

The non-volatile products obtained after filtering and distilling off the benzene are dissolved in chloroform. The benzene distillate contains considerable quantities of quinone. The non-volatile products can be seen to contain yellow needles of crystalline quinone which can be

picked out (m.p. 114°–115°, mixed m.p. with quinone 114°–115°). Separation was performed by thin-layer chromatography.

The main product is diphenyl picrylhydrazine, m.p. 169°–170°, mixed m.p. with diphenyl picrylhydrazine 169°–170°, and in most runs the amount of it corresponds to a reduction of 75 per cent of the radical. This compound results from self-reduction of the hydrazyl radical while the quinone results from simultaneous oxidation of another molecule of the radical in consecutive steps. 2′,4′,6′-trinitro-4-aminodiphenylamine (picryl phenylenediamine) is isolated from one of the bands almost pure; usually about 0·10 g, m.p. 142°–143° (found: C, 45·65, 45·83; H, 2·90, 2·67; N, 21·50, 21·24; mol. wt. (Rast 290); calculated for $C_{12}H_9N_5O_6$: C, 45·15; H, 2·84; N, 21·94; mol. wt., 319).

The only obvious way to make this compound is by the action of picryl chloride on phenylenediamine, but this has been shown to yield a high melting product.

According to Wedekind[1], the product is an addition product (found: C, 40·90; H, 3·14; N, 19·91). He states that this corresponds to the addition product $C_{12}H_{10}N_5O_6Cl$ (calc. C, 40·48; H, 2·81; N, 19·73). Wedekind's work was repeated by Morgan and Micklethwait[2]. The product has a melting point of 185°–187° and forms a sparingly soluble salt with strong hydrochloric acid, but they give no analysis.

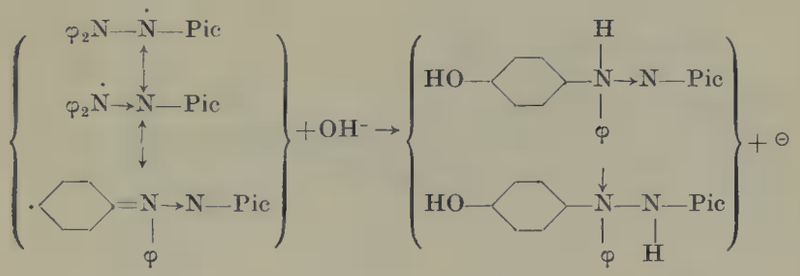
A consideration of the possible resonance forms involving the para groups would lead one to expect that the salt would be unstable. Attempts to hydrolyse the compound, m.p. 142°–143° (which analyses for *N*-picryl phenylenediamine), to its components yielded a mixture of alkali and acid insoluble products.

The following series of reactions is one explanation of what has obviously happened.

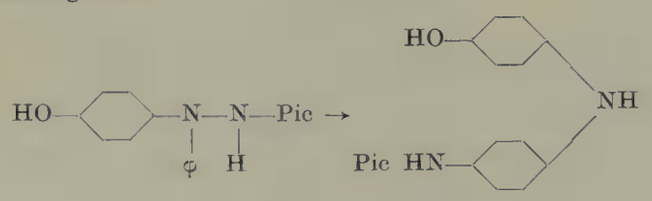
Reduction:

(Pic≡Picryl)

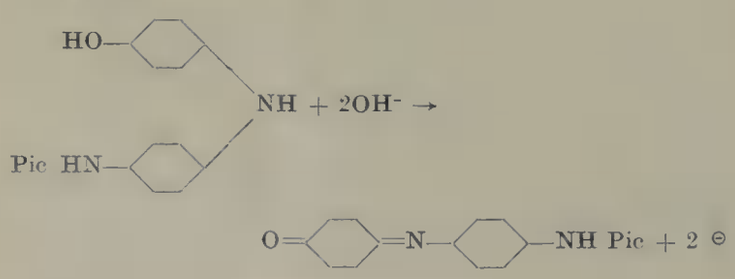
Oxidation:



Rearrangement:



Oxidation:



The two oxidation steps require two further moles of hydrazyl yielding a product which is the *N*-picryl substituted indanilin. Hydrolysis yields quinone and *N*-picryl phenylenediamine as found. It could also yield picramid and *p*-aminophenol. If the first oxidation step involves the ortho position the indanilin then has an ortho-quinoid linkage and would yield orthoquinone, a difficult substance to isolate. If the rearrangement (or equivalent) of the *p*-hydroxydiphenyl picryl hydrazine involves the ortho position of the phenyl group, processes involving a cyclic intermediate are possible. Likewise the *p*-hydroxy diphenyl picryl hydrazine could be oxidized to the substituted hydrazyl. The total amount of *N*-picryl diphenylamine, if all the reaction went as outlined, should be 0·4 g.

The reaction occurs very slowly on sulphuric-acid-free TiO_2 (anatase) and silica gel. It occurs rapidly in sulphuric acid saturated benzene. On 'Carbolac 2' without any addition of acid or water the reaction occurs at one-fifth the rate of that on the anatase (7·5 per cent sulphuric acid), yielding the same products.

During the reaction on the anatase no sulphuric acid was removed. Results to be reported elsewhere show that on all surfaces the reaction is first order in the radical. This indicates a mechanism involving mobile *F*-centres or the equivalent as the only possibility.

The triggering reaction must be the addition of an electron to the hydrazyl in the reduction part, since the oxidation part involves consecutive reactions. On the anatase the reaction yielding the electron is:

$$TiX_3 + H_2O \rightarrow Ti(OH)X_3 + H^+ + \ominus$$

where X represents any group covalently linked to titanium. The reaction taking the electron in the oxidation part is:

$$Ti(OH)X_3 + \ominus \rightarrow TiX_3 + OH^-$$

These two reactions yield a stoichiometric balance with the reduction and oxidation equations written here. The first involves the destruction of an *F*-centre which must appear elsewhere if the surface is mobile. The electron to produce an *F*-centre comes from the oxidation step.

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Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections

The classic Polanyi flame work[1,2] explored the reactions of sodium atoms with a very wide range of halogen compounds. For some of these the rate constants were found to be extremely large ($\sim 10^{14}$ c.c. mole^{-1} sec^{-1}), corresponding to "reaction on more than every gas-kinetic collision". This was attributed to an electron-jump mechanism,

$$Na + X - R \rightarrow Na^+ — X^- — R \rightarrow Na^+X^- + R$$

Here essentially all collision trajectories that pass within the radius r_c at which formation of the ion-pair becomes possible lead to reaction[3]. This radius is given by $e^2/r_c = I(Na) - E^v(X-R)$, where I denotes the ionization potential of the alkali atom and E^v the vertical electron affinity of the halogen molecule. Recently, several alkali atom reactions of this type (with $X-R = Br_2$, ICl, PBr_3, CBr_4, $SnCl_4$, SF_6, etc.) have been examined in molecular beam scattering experiments[4]. The form of the angular distribu-

tions of elastic and reactive scattering and other detailed dynamical properties observed in these experiments can be very plausibly interpreted in terms of the electron-jump mechanism.

However, a much larger class of 'reactions with inertia' was also examined in the Polanyi sodium flame experiments. These cover a broad spectrum of reactivity and many proceed at rates which are $\sim 10^4$ times slower than reaction at every collision[1,2]. The simple electron-jump mechanism cannot apply to these reactions, as the cross-sections correspond to such small values of r_c that other chemical interactions must become very important. It is therefore of particular interest to note a simple correlation which indicates that even for this class of reactions the electron affinity of the reactant molecule plays a dominant part.

Recently, Lovelock has estimated electron absorption coefficients for many compounds from gas chromatography[5]. Under the conditions of measurement this coefficient is a thermodynamic parameter. It governs the thermal equilibrium between electrons, ions, and neutral molecules and is essentially a measure of the adiabatic electron affinity[6]. (The difference between the vertical and adiabatic electron affinity becomes irrelevant for reactions with small values of r_c as the bond distances in the transition state are expected to be considerably displaced from the equilibrium values in the isolated reactant molecule.) The electron absorption coefficients show systematic variations with chemical structure which Lovelock has mapped out. His results are remarkably similar to those found for Na atom reaction rates in the Polanyi experiments. For example, the following factors markedly enhance both capture of thermal electrons and reaction with Na atoms: (1) change of the halogen atom, in the order F→Cl→Br→I; (2) presence of an oxygen atom in addition to the halogen; (3) multiple substitution which increases the number of electrophoric groups or brings them closer together; (4) location of the halogen atom at an allylic or benzylic position (double bond in β-position to X atom). Also, the rates for both processes are depressed when (5) the halogen atom is adjacent to an ethylenic double bond or a benzene ring (double bond in α-position to X atom).

Only order-of-magnitude values are known for both the electron absorption coefficients and the Na reaction rate constants. However, the correlation holds for a wide variety of compounds and in the case of factors (1) and (3) covers a range of $\sim 10^5$ in reactivity. Fig. 1 gives a comparison for a few chlorinated hydrocarbons and illustrates the operation of factors (3)–(5). The values given for the electron absorption coefficients[5] are relative to that for chlorobenzene. Approximate absolute values can be assigned since the cross-section for chlorobenzene has been estimated[7] to be $\sim 0{\cdot}1$ to 1 Å^2. Thus an absorption coefficient of 10^3 corresponds to a cross-section of about 100–1000 Å^2. For the Na reactions the cross-sections can be roughly estimated from the ratio of the rate constant to the average thermal velocity; a rate constant of 10^{14} corresponds to a cross-section of about 30 Å^2.

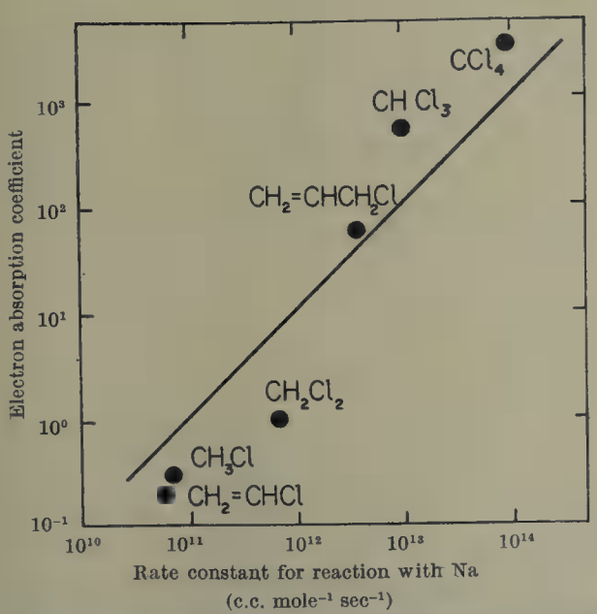

Fig. 1. Correlation between electron absorption coefficients (at 100° C) and rate constants for reaction with sodium atoms (at 250° C). The line is included merely to indicate a slope of unity. The values of the electron absorption coefficients are relative to that for chlorobenzene, which is taken as unity

The extended parallelism between electron affinity and reactivity indicated by this correlation is perhaps to be expected from familiar arguments concerning stabilization of the transition state by charge delocalization[8,9]. For these simple gas phase reactions, it seems likely that the factors (1)–(5) are mainly governed by delocalization. Although these factors sometimes operate in a qualitatively similar way for reactions in solution, they are often overcome by other effects[9].

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A Preparation of Cementite

Several methods for the preparation of the carbides of iron have been reported in the literature. In order to obtain a satisfactory product it may be necessary to use a relatively high temperature[1], or long periods of reaction during which reaction conditions must be controlled within narrow limits for several weeks[2,3]. Using these methods we found considerable difficulty in preparing a sample of iron carbide which was sufficiently pure to be used in a projected study. During preliminary investigations of reactions of transition metal salts of organic acids, however, it was noticed that the solid residue from the thermal decomposition in vacuum, at about 500° C, of a ferric salt of mellitic acid gave an X-ray diffraction pattern characteristic of cementite. This communication is concerned with the use of this compound for the preparation of cementite. A detailed report of the kinetics of the thermal decomposition reaction will be published elsewhere[4]. Studies of the thermal decomposition of ferric salts of benzoic and of phthalic acids showed that no detectable cementite was present in the non-volatile crystalline products[5].

For use in the preparation of cementite the ferric salt of mellitic acid was formed by heating two moles of ferric hydroxide with one mole of mellitic acid in a little water at 90° C for 30 min. The ferric hydroxide had been freshly precipitated from ferric chloride solution by the addition of ammonium hydroxide (both 'AnalaR'), boiled and washed by decantation six times. For the present study five preparations of ferric mellitate were investigated, in which the tan-coloured precipitates formed as above were treated as follows:

Sample *A*, filtered immediately after cooling, but not washed.

Samples *B*1 and *B*2, washed by decantation; the precipitate remained tan coloured.

Samples *C*1 and *C*2, the wet product washed with distilled water on the filter paper. Part of the product darkened, suggesting that some hydrolysis occurred.

Each batch of material was dried and the elements determined by combustion analyses. Iron was calculated from the weight of the residue from ignition in oxygen, which was assumed to be Fe_2O_3 only, and oxygen was found by difference. Results are shown in Table 1. The relatively large iron content of samples *C*1 and *C*2 is consistent with the foregoing view that some hydrolysis of the product has occurred, possibly yielding some ferric hydroxide.

Table 1. Analyses of Iron Salt of Mellitic Acid

Sample	Fe	C	H	O
A	20·78	24·91	2·57	51·74
*B*1	20·45	24·61	2·53	52·41
*B*2	20·84	23·91	2·64	52·61
*C*1	23·51	24·08	2·48	49·93
*C*2	24·06	24·13	2·52	49·29

The non-volatile products remaining after thermal decomposition of samples of the foregoing salts between 470° and 500° C *in vacuo* or in contact with the gaseous products (excluding water) were pyrophoric, and considerable difficulty in handling was experienced. Products from decompositions between 450° and 470° C did not so readily oxidize in air. Decomposition of samples of salts *C*1 and *C*2 gave solid products which X-ray examination showed to contain appreciable amounts of Fe_2O_3 and/or Fe_3O_4 together with a diffuse pattern attributable to cementite.

Samples of preparations *A*, *B*1 and *B*2, when decomposed for intervals between the limits 6 h at 460° C and 1 h at 500° C, gave cementite as the main crystalline product, but this was only observed for those samples in which atmospheric oxidation was prevented by chilling the solid to −195° C before admission of air to the apparatus, and subsequent storage under benzene. Samples for X-ray investigation were thoroughly mixed with a glue before evaporation of the benzene. Small traces of oxide were detected in the products from those reactions in which the gaseous decomposition products were allowed to remain in contact with the reactant during decomposition.

Samples of cementite, having X-ray diffraction patterns almost identical with a sample of cementite supplied by Dr. L. J. Dry of the S.A. Coal, Oil and Gas Corporation, Ltd., Republic of South Africa, were obtained by decomposition of 15 mg of sample *B*1 and of 2 g of sample *B*2 at 480° C with continual evacuation of products. Both samples were pyrophoric and an attempt was made to prevent atmospheric oxidation by the methods already described.

Determination of the surface area of the product cementite, from measurements of the adsorption of nitrogen at −195° C and application of the Brunauer–Emmett–Teller equation, showed that both sample *B*1, after decomposition 2 h 480° C with accumulation of gaseous products, and sample *A*, after decomposition 2 h 470° C with continual evacuation, had surface areas 240 ± 10 $m^2 g^{-1}$. Due to difficulties in handling in air we cannot be certain that no oxidation of these samples occurred, but both results show that a high surface area product is given.

The detailed mechanisms of the decomposition reactions are discussed elsewhere[4], and only particular points arising from the results presented here will be considered:

(1) The decomposition product contains excess carbon; the composition is believed to be Fe : C : : 1 : 2·6. It is probable that a layer of finely divided carbon over the crystallite surfaces reduces sintering, so stabilizing the high-area product observed.

(2) The product from decomposition in the presence of gaseous oxides of carbon was indistinguishable from that observed from decomposition with continual evacuation. It seems probable, therefore, that the carbide was formed by the interaction of the finely divided iron–carbon mixture (the latter being the non-volatile product from the aromatic ring present in the acid radical) rather than a mechanism in which finely divided iron reacted with gaseous carbon monoxide.

(3) It is suggested[4] that the decomposition of the ferric salt of mellitic acid 400°–500° C may involve reaction between a carbonaceous residue and an iron oxide. The foregoing results for samples *C*1 and *C*2, where it is believed that ferric oxide was present in the reactant, suggest that the presence of this oxide may modify the course of the decomposition reaction to yield a product containing an appreciable amount of oxide. The mechanism described[4], however, may be applicable to samples containing lower oxides of iron where ferric hydroxide was absent from the reactant.

Cementite may therefore be prepared by the method outlined here. Two disadvantages of the method are that the product contains excess carbon (Fe : C : : 1 : 2·6) and that the pyrophoric material is difficult to handle. Advantages of the method are that cementite may be prepared relatively rapidly at a low temperature (namely, < 500° C) in a glass apparatus.

One of us (J. F.) held a postgraduate studentship awarded by the Ministry of Education, Northern Ireland.

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Oxidation of Some 2,4-Dinitrophenylamines with Ceric Salts

During an investigation into the mechanism of cerium-initiated graft polymerization of acrylic acid on to nylon, the behaviour of the dinitrophenylated fibre led us to examine the oxidation with ceric salts of some model 2,4-dinitrophenylamino-compounds. Since we are unable to complete the work, we are reporting the preliminary results, which may arouse interest elsewhere in the possible scope and mechanism of this reaction. The compounds examined were the potassium salts of DNP-taurine (I, m.p. 249°–252° C) and 3-DNP-aminopropanesulphonic acid (II, m.p. 231°–233° C), the base (III, m.p. 71°–73° C) and its quaternary derivative (IV, m.p. 276°–277° C), obtained in 70 per cent yield by treatment of III with 1,3-propanesultone in boiling acetonitrile for 7 h; they all gave satisfactory analyses.

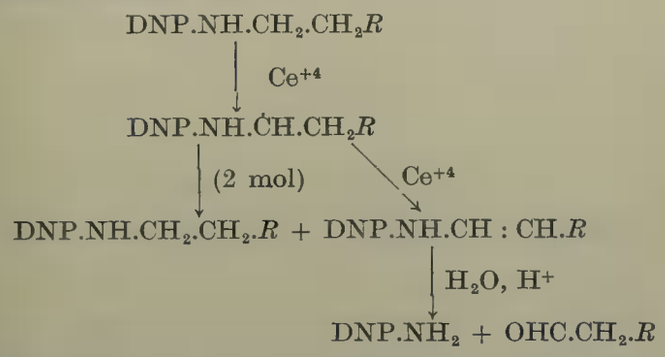
$DNP.NH.CH_2.CH_2.SO_3K$ (I) $DNP.NH.CH_2.CH_2.CH_2.SO_3K$ (II)

$DNP.NH.CH_2.CH_2.NMe_2$ (III)

$DNP.NH.CH_2.CH_2.\overset{+}{N}Me_2(CH_2)_3\overset{-}{SO_3}$ (IV)

Oxidation at room temperature of each DNP-amine (0·01 g-mol) in water (50 ml.) or, for III, N sulphuric acid (50 ml.) with a solution of ceric ammonium sulphate (0·03 g-mol.) in N sulphuric acid (100 ml.) rapidly gave a yellow precipitate, which was isolated after 1 h and shown to be 2,4-dinitroaniline by comparison of its m.p. and infra-red spectrum with those of an authentic specimen. The yields were 95, 88, 75 and 70 per cent, respectively. Limiting the amount of oxidant to 1 mol per mol DNP-amine resulted in only an approximate halving

of the yield of 2,4-dinitroaniline and no intermediate product was detected. Taurine did not reduce ceric ammonium sulphate significantly in 7 days at 25° C. Rates of oxidation at 25° C (Fig. 1) were determined by treating the compounds (0·001 g/mol), dissolved in water or N sulphuric acid (50 ml.), with ceric ammonium sulphate (0·005 g mol) in N sulphuric acid (50 ml.) and titrating aliquots (10 ml.) of the reaction mixture with 0·05 N ferrous ammonium sulphate at suitable intervals. These curves indicate that the rapid primary oxidation yielding 2,4-dinitroaniline requires two equivalents of cerium, and if an analogy can be drawn with the oxidation of alcohols by ceric salts[1] or of alkylamides by persulphates[2] one possible reaction path involves initial formation of a radical by abstraction of hydrogen at the α-carbon atom; disproportionation of this yields an enamine, which is then hydrolysed. Alternatively the enamine may result from direct attack of the radical by a second cerium ion:



Since the solutions were not de-aerated, routes involving oxidation of a peroxide derived from the initial radical and oxygen could also be implicated. Further, slow oxidation of the aldehyde would account for consumption of cerium beyond two equivalents.

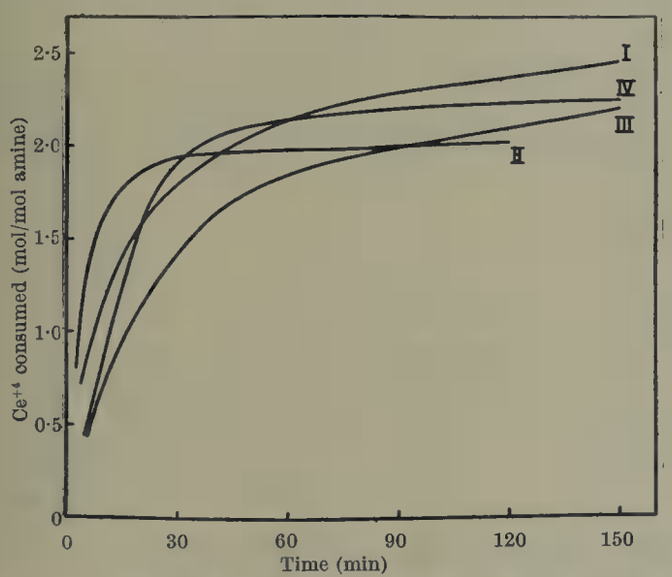


Fig. 1. Oxidation of the 2,4-dinitrophenylamines I–IV with ceric ammonium sulphate at 25° C

The solutions obtained from oxidation of compounds I–III with 3 equivalents of ceric ammonium sulphate, after removal of dinitroaniline and cerium salts, on treatment with 2,4-dinitrophenylhydrazine in 5 N hydrochloric acid at 25° C yielded derivatives which, after recrystallization, melted with decomposition at 239°–244°, 202°–203° and 158°–159° respectively, and gave elemental analyses differing greatly from those of the expected aldehyde 2,4-dinitrophenylhydrazones. However, a concentrated solution from oxidation of compound (III) gave glyoxal *bis*-2,4-dinitrophenylhydrazone when boiled with the reagent in N hydrochloric acid, as did the authentic diethyl acetal of dimethylaminoacetaldehyde[3], thus affording some confirmation that this aldehyde is a product of the oxidation.

We thank Dr. H. R. Cooper and Mr. B. F. Sagar for their advice.

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BIOCHEMISTRY

Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells

In recent years several attempts have been made at a complete analysis of metabolic pathways. In comparing complex systems, be they intact cells or homogenates, with isolated enzymes, both similarities and differences[1–3] have been found. So far no single pathway has been completely elucidated with respect to its kinetics. For this reason we turned to the erythrocyte, as representing both structurally and metabolically one of the simplest biological systems. One line of research which is to be reported here was concerned with the regulation of glycolysis on the level of lactate oxido-reductase. These studies were extended to ascites tumour cells. In view of the generally high activity of the lactate oxido-reductase (LDH) it may be assumed that the lactate/pyruvate system is at equilibrium within the cell[1]. Recently, however, deviations from the expected lactate/pyruvate ratios have been observed in brain homogenates[3]. We explored this question in a more detailed manner by studying the effect of *p*H on the formation of pyruvate and lactate.

The pH dependence of the lactate/pyruvate ratio. From the stoichiometry of the LDH reaction one would expect a *p*H dependence of the lactate/pyruvate ratio such that with increasing *p*H this ratio is diminished. Table 1 shows that the experimental results do not correspond to this assumption. As a matter of fact, in erythrocytes just the reverse is observed. For further study of these paradoxical observations the *p*H dependence of the lactate/pyruvate ratio of stroma-free dialysed haemolysates and of particle-free homogenates of ascites tumour cells was studied. The formation of pyruvate from added NAD and lactate was determined at varying *p*H values. The amounts of pyruvate observed were compared with calculated values based on the equilibrium constant of LDH. The results were the same as with intact cells. While there was approximate agreement between expected and experimental pyruvate formation at *p*H 8·4, twenty times the expected value of pyruvate was formed at *p*H 6·4. To explain these results we postulated the existence of an oxidation factor in erythrocytes and ascites tumour cells which disturbs the lactate/pyruvate equilibrium particularly at lower *p*H-values.

$NADH_2$- and $NADPH_2$-oxidases in erythrocytes and ascites tumour cells. Further experiments have indeed demonstrated, both in erythrocytes and in ascites tumour cells, extramitochondrial enzymes which oxidize $NADH_2$

Table 1. Lactate/Pyruvate Ratio in Erythrocytes and Ascites Tumour Cells

*p*H	10^{-3} moles/l. Lactate		Pyruvate		Lactate/Pyruvate	
	Eryth.	Ascites tumour cells	Eryth.	Ascites tumour cells	Eryth.	Ascites tumour cells
6·4	2·57	28·3	0·148	1·47	17·3	19·2
7·0	—	30·6	—	1·34	—	22·8
7·4	3·03	35·5	0·089	1·30	33·7	27·3
8·0	—	34·5	—	1·08	—	31·9
8·4	4·86	—	0·033	—	148·0	—

Human erythrocytes were incubated with 0·2 M *tris*-maleate buffer. Ascites tumour cells were incubated in Ringer-phosphate-bicarbonate solution at 3·75° C. The glucose concentration was 250 mg/100 ml.

Table 2. Oxidase Activities with $NADH_2$ and $NADPH_2$ in Erythrocytes and Ascites Tumour Cells

	Enzyme activity 10^{-6} moles/l.cells/min	
	$NADH_2$	$NADPH_2$
Human erythrocytes	0·102	0·177
Rabbit erythrocytes	0·113	0·099
Ascites tumour cells	0·017	0·018

The oxidases were measured spectrophotometrically at 340 nm, after 48 h dialysis in 0·1 M phosphate buffer, *p*H 6·4.

and $NADPH_2$ (Table 2). In Fig. 1 the *p*H-dependence of the erythrocyte oxidases is shown in a haemoglobin-free haemolysate of rabbit erythrocytes. It may be seen that both enzyme activities are optimal at *p*H 6·4. The $NADH_2$ oxidase activity is inhibited by higher concentrations of its substrate; with 1×10^{-4} M $NADH_2$ inhibition was practically complete. The inhibitory effect of $NADPH_2$ on its oxidase was much less pronounced. Under anaerobic conditions the oxidase activities were low whereas cyanide had no effect. From these observations it would appear that the oxidases are flavin enzymes. Formerly the existence of $NADH_2$- and $NADPH_2$-dehydrogenases (diaphorases) had been demonstrated in haemolysates[11]. It might well be that the oxidases here described, and the dehydrogenases, are identical. On the other hand, it could be demonstrated that the oxidation of $NADH_2$ and $NADPH_2$ is mediated by at least two different enzymes. From the results in Table 3 it may be seen that a significant separation of the two activities could be obtained, so that a more than twenty-fold shift in the oxidase activity ratios of $NADH_2$ and $NADPH_2$ could be obtained. The two enzyme activities also differed in their stability towards lyophilization and dialysis, the $NADPH_2$ oxidase being the more sensitive. The dehydrogenase activities resembled the oxidases. The activity of these ratios was shifted about fifty-fold by the fractionation. The ratio of the oxidase to diaphorase activity was significantly higher with $NADPH_2$ as compared with $NADH_2$. Further purification of the enzymes is in progress.

The lactate-NADP-oxidoreductase in erythrocytes and in ascites tumour cells. The literature contains several reports concerning the reactivity of LDH of different tissues with NADP. With purified enzymes, various ratios of activity with NADP as compared with NAD have been reported, ranging from 0·003 to 0·05. In other communications the importance of this reaction for the generation of $NADPH_2$ for biosynthesis has been assessed[4,5]. We have performed similar experiments on erythrocytes and ascites tumour cells. Table 4 summarizes the data from the literature together with our own results. It may be seen that erythrocytes have the highest activity with NADP which has been observed so far. The variation in the activity of the LDH with the two coenzymes led us to search for an NADP-specific LDH. In Table 5 are shown the results of a preliminary separation on DEAE cellulose. Whereas the original ratio of NAD/NADP activities in the dialysate of erythrocytes was 10:1, it increased to 60:1 in the first eluate and was only 1:2 in the second fraction.

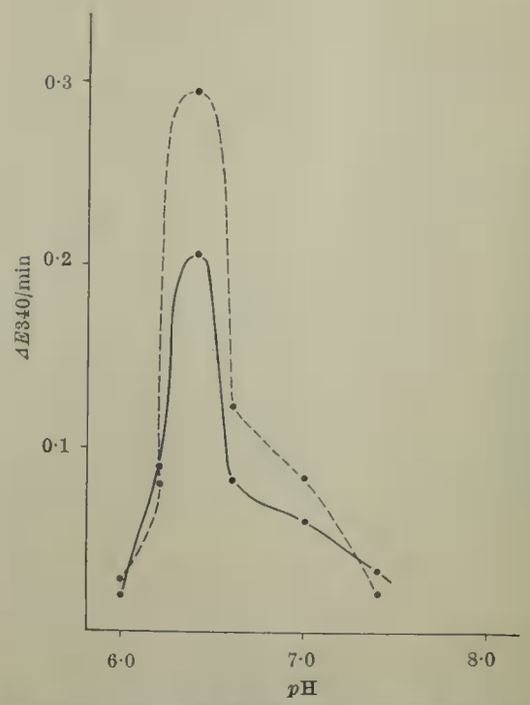

Fig. 1. *p*H-dependence of $NADH_2$ (——) and $NADPH_2$ oxidase (– – – –) in erythrocytes. 0·1 ml. of eluate (DEAE cellulose column) from stroma-free haemolysate from rabbit erythrocytes, 3·0 ml. of a 0·1 M phosphate buffer and 0·02 ml. of $NADPH_2$ solution, final concentration 5×10^{-5} moles per cuvette

In Fig. 2 are shown the *p*H optima of the two enzyme activities in erythrocytes. Whereas the main optimum of the NADP-dependent LDH is located at about *p*H 6·0, two optima were observed with NAD; the main optimum was at about *p*H 9·0 and a second one at about *p*H 5·0. In a dialysate of ascites tumour cells (Fig. 3), the *p*H optimum of the NADP-dependent LDH was also at *p*H 5·8–6·0 and that of the NAD-dependent enzyme at *p*H 8·0.

It is conceivable that the acid *p*H-optimum represents an isoenzyme of the NAD-dependent LDH.

From these results it would appear that an NADP-dependent LDH occurs generally and accounts for the

Table 4. Lactate Oxydoreductase with NADP and NAD

	NAD	NADP	NADP/NAD	
Erythrocytes	8·50	0·72	0·085	
Ascites tumour cells	41·70	1·00	0·024	
Rabbit muscle	—	—	0·0057	(7)
Bovine heart muscle	—	—	0·01–0·003	(8)
Cattle retina	—	—	0·05	(9)
Bovine cornea	—	—	0·005	(10)

1 enzyme unit = 1×10^{-6} mole/l./min.

Table 5. Lactate Oxydoreductase in Haemolysate of Rabbit Erythrocytes after DEAE-cellulose Fractionation

Vol. (ml.)	Proteins (mg)	Enzyme units/vol. NAD	NADP	Specific act. $\times 10^{-3}$ NAD	NADP	NAD/NADP	
70	6,195·0	15·2	1·45	2·5	0·24	10	Stroma-free haemolysate (dialysate)
240	98·6	49·3	0·83	499·0	8·4	60	Fraction I
600	126·0	0·24	0·51	2·0	4·0	0·5	Fraction II

1 unit = 1×10^{-6} mole/ml./min.

Table 3. Oxidase and Dehydrogenase (Diaphorase) Activity in Eluates from a DEAE Cellulose Column of a Haemolysate

Volume (ml.)	Protein (mg)	Coenzyme	Units/vol.* Oxidase	Units/vol.* Diaph.	Specific activity Oxidase	Specific activity Diaph.	$NADH_2/NADPH_2$ Oxidase	$NADH_2/NADPH_2$ Diaph.	
70	6,195·0	$NADH_2$	—†	63·70	—	0·010	—	94·0	Dialysate
		$NADPH_2$	—	0·67	—	0·0001			
240	98·6	$NADH_2$	34·2	38·40	0·35	0·39	1·6	9·0	Fraction I
		$NADPH_2$	21·6	4·30	0·22	0·044			
600	126·0	$NADH_2$	7·1	12·00	0·057	0·095	0·075	1·7	Fraction II
		$NADPH_2$	93·8	7·20	0·11	0·057			
71	28·1	$NADH_2$	17·3	14·70	0·62	0·53	2·2	12·0	Fraction Ia
		$NADPH_2$	7·8	1·26	0·28	0·045			

* 1 oxidase unit = 10^{-3} moles/ml./min. 1 diaphorase unit = 10^{-6} moles/ml./min.

† The activity in the dialysate of the stroma-free haemolysate could not be measured because of the presence of haemoglobin. The haemoglobin was eluated from the column with 0·002 M phosphate buffer, *p*H 7·4, whereas fractions I and II were eluated with 0·2 M buffer at the same *p*H. Then Fraction I was lyophilized and dialysed again and the activity determined. Diaphorase activities were measured with dichlorphenolindophenol and $K_3Fe(CN)_6$ as acceptors.

varying activities with NADP reported in the literature. This enzyme plays a definite part in the metabolic regulation of the cell. The importance of provision of $NADPH_2$ for various reductive syntheses has been stressed[4]. The NADP-dependent LDH is probably the specific mediator for the reduction of NADP by lactate. On the other hand, an oxidation of $NADPH_2$ by pyruvate may also have a regulatory significance. Another aspect of its function is that of a cross-link between the Embden–Meyerhof and the oxidative pentose pathways.

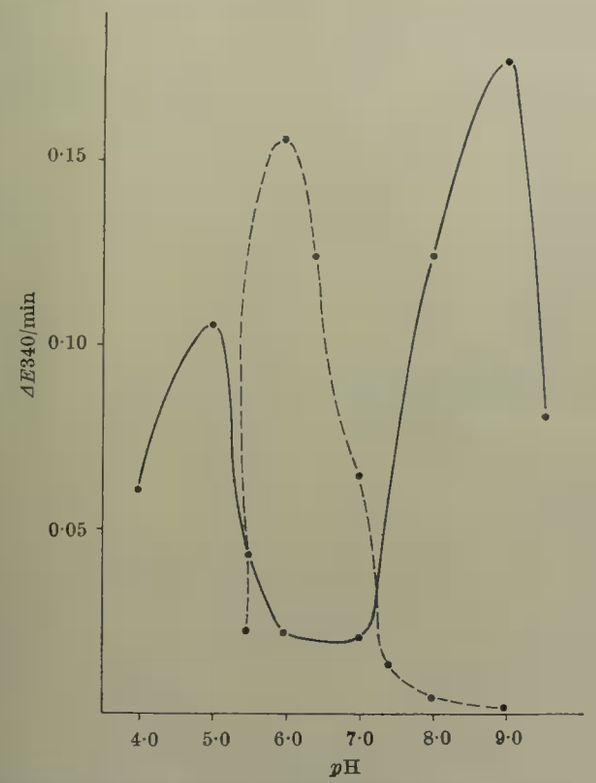

Fig. 2. *p*H-dependence of LDH in erythrocytes. 0·1 ml. of eluate NADP–LDH/3·2 ml. total volume 0·05 ml. of eluate from NAD–LDH (1:10)/3·2 ml. total volume. For the range of *p*H 4—5 5, 0·1 M citrate was employed. 0·1 M phosphate buffers of varying *p*H were used. The final coenzyme concentration was 5×10^{-3} M. ——, $NADH_2$–LDH; – – – –, $NADPH_2$–LDH

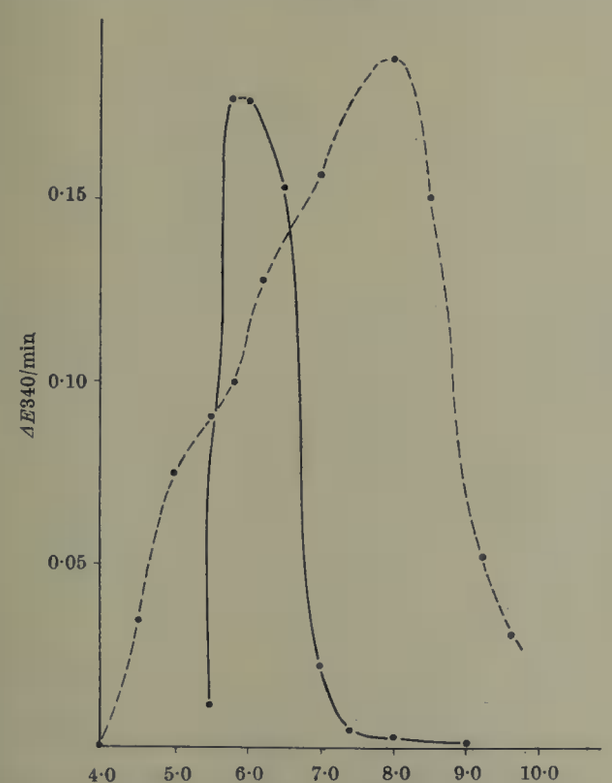

Fig. 3. *p*H-dependence of LDH in ascites tumour cells. Experimental conditions as described for Fig. 2. ——, $NADPH_2$–LDH; – – – –, $NADH_2$–LDH

Relating to the outset of our investigations, an explanation of the deviations of the lactate/pyruvate ratio from the theoretical values has been found in the activities of 2 types of enzymes: (1) the $NADH_2$ and $NADPH_2$ oxidases, and (2) the NADP-dependent LDH. In addition we have recently found an LDH which is independent of a pyridine nucleotide in ascites tumour cells[6].

The effect of the oxidases is probably the more important one of the two. The oxidases represent part of the extramitochondrial oxygen uptake of the cells. For erythrocytes at least, their contribution may be of some importance at lower *p*H values.

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Effect of *Trans*-Solutes on the Fluxes of Chloride Ions across Artificial Membranes

In free diffusion across membranes the unidirectional fluxes of a solute are usually treated as mutually independent movements: each flux being a function only of the electrochemical potential of its solute in the compartment from which it originates (*cis* side). Across biological membranes, however, such as the gastric mucosa of the frog[1], or some cellular membranes[2–4], the fluxes of various solutes strongly depend on the nature of the solutes on the *trans* side. These *trans* effects have been interpreted in terms of a carrier exchange mechanism with different affinity to different ions and molecules[5,2]. In the present work such *trans* effects have been studied in artificial membranes, in which transport or exchange carriers can be excluded.

Commercial packing 'Cellophane' or dialysing membranes were mounted between 'Lucite' chambers, as described earlier[1]. In order to keep the experimental conditions comparable to those with the gastric mucosa of the frog, the *cis* side, to which the radioactive material was added, was bathed by (frog) Krebs bicarbonate ringer ('nutrient') solution (84·6 mM NaCl, 3·2 mM KCl, 1·8 mM $CaCl_2$, 0·8 mM KH_2PO_4, 0·8 mM $MgSO_4$, 17·8 mM $NaHCO_3$). For the control experiments the *trans* side was bathed with 'secretory' solution (102·1 mM NaCl, 4·3 mM KCl, 1·8 mM $CaCl_2$, 0·8 mM $MgSO_4$). In the test experiments the chloride of the *trans* solution was equivalently replaced by other anions. In the case of di- and trivalent anions iso-osmolarity was restored by mannitol In some experiments, all but 10·2 mM of the NaCl was. replaced by iso-osmolar concentrations of non-electrolytes. Both solutions were bubbled by 5 per cent CO_2 and 95 per cent O_2. The unidirectional fluxes were measured with radioactive tracers as described elsewhere[1], and corrected for variations of self-absorption.

Contrary to the assumption of free diffusion the flux of chloride ions across artificial membranes decreased if the chloride ions of the *trans* side were replaced by heavier ions or molecules (Figs. 1 and 2). The effect seems to be an almost linear function of the cube root of the *trans* particle weight.

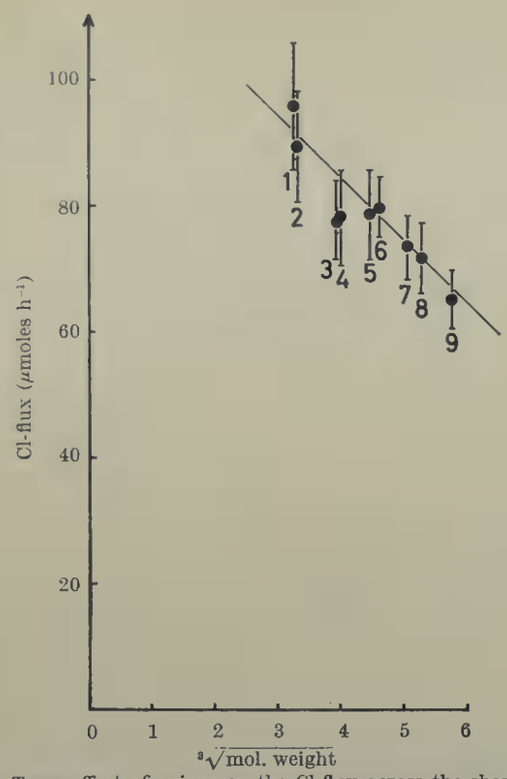

Fig. 1. *Trans* effect of anions on the Cl-flux across the short-circuited 'Cellophane' membrane. Composition of the solutions is given in the text. The *trans* ions are; 1, Cl; 2, formate; 3, HCO_3; 4, NO_3; 5, pyruvate; 6, SO^{4-}; 7, I^-; 8, tartrate; 9, citrate. Room temperature. Correlation coefficient $r = 0{\cdot}76$ ($P < 0{\cdot}001$)

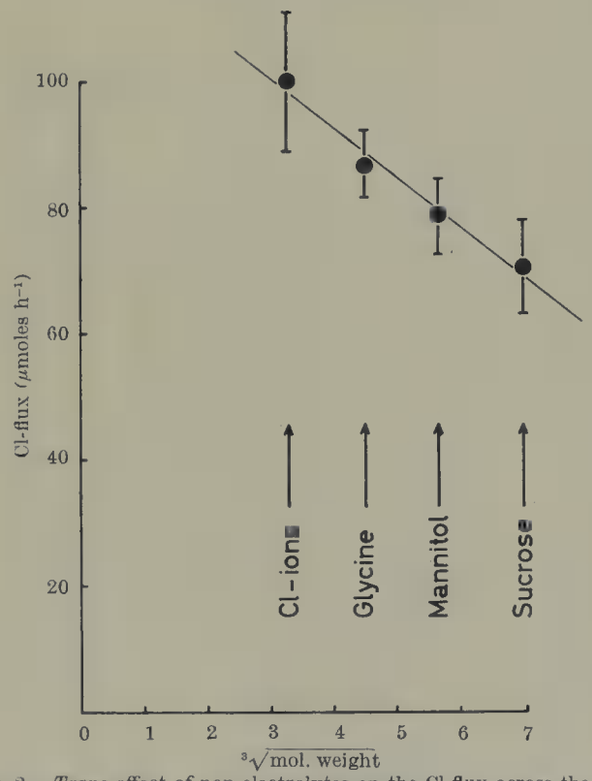

Fig. 2. *Trans* effect of non-electrolytes on the Cl-flux across the short-circuited 'Cellophane' membrane. Composition of the solutions is given in the text. The *trans* solutes used are indicated below. Room temperature. Correlation coefficient $r = -0{\cdot}88$ ($P < 0{\cdot}001$)

These *trans* effects are scarcely due to changes of the hydration of the membrane. Although dialysing membranes seem to take up slightly less water if the solute weight of the bathing solutions increases, these differences scarcely exceed 5 per cent of the control and may be still smaller if the *trans* solution, as in the flux experiments, bathes only one side of the membrane (Table 1). Furthermore, no correlation was found for a given *cis–trans* combination of solutes between the *trans* effect and the accidentally varying hydration of different membranes.

The *trans* effects cannot be accounted for by changes of viscosity within the membrane pores either, since the

Table 1. Effect of Various Electrolytes and Non-electrolytes on Water Content of Dialysing Membrane

Bathing solution	No. membranes tested	H_2O content ml.g^{-1} (dry weight)
110 mM NaCl	3	1·26 ± 0·05
55 mM Na_2SO_4 + 55 mM Mannitol	3	1·25 ± 0·04
55 mM Na_3Citrate	3	1·22 ± 0·06
220 mM Glycerol	4	1·30 ± 0·06
220 mM Mannitol	4	1·24 ± 0·03
220 mM Sucrose	4	1·21 ± 0·05

Pieces of dialysing membrane (No. 4465–A_2) were bathed for 1 h in the solution at room temperature. The H_2O content was determined by weighing before and after evaporation (at 105°) to dryness with a correction for solute residues.

Table 2. Effect of Various Non-electrolytes on the Conductivity of 'Cellophane' Membranes for 10 mM NaCl

Bathing solution	Conductances 2 sheets	4 sheets mmhos per 0·785 cm²	6 sheets
170 mM Glycerol + 10 mM NaCl	8·0 ± 0·3	4·1 ± 0	2·9 ± 0
170 mM Mannitol + 10 mM NaCl	8·0 ± 0·3	4·3 ± 0	2·9 ± 0
170 mM Sucrose + 10 mM NaCl	7·9 ± 0·2	4·1 ± 0	2·7 ± 0

All measurements were made at room temperature with three different membranes. In order to amplify the effects each membrane was made up of 2, 4 or 6 sheets of packing 'Cellophane'.

conductance of NaCl solutions decreased only slightly with increasing weight of added non-electrolyte molecules (Table 2).

According to Onsager's principles the flux of a given solute across the membrane may be altered by interaction with other fluxes, for example with the solvent flux in bulk flow[6]. Even between iso-osmolar solutions bulk flow might result if *cis* and *trans* solutes differ with respect to either the osmotic or the Stavermann deflexion coefficient. The osmotic coefficients of the binary salts, as derived from the molar freezing point depressions, are rather close to those of NaCl[7]. Those of the non-electrolytes, while very similar to each other, are all greater than that of NaCl, so that their effect on Cl-fluxes, if any, would be in the 'wrong' direction. The Stavermann coefficients, on the other hand, increase with the particle size[8]. Any bulk flow resulting from differences in these coefficients should obviously be directed towards the side with the larger particles, that is, also in the 'wrong' direction. Hence bulk

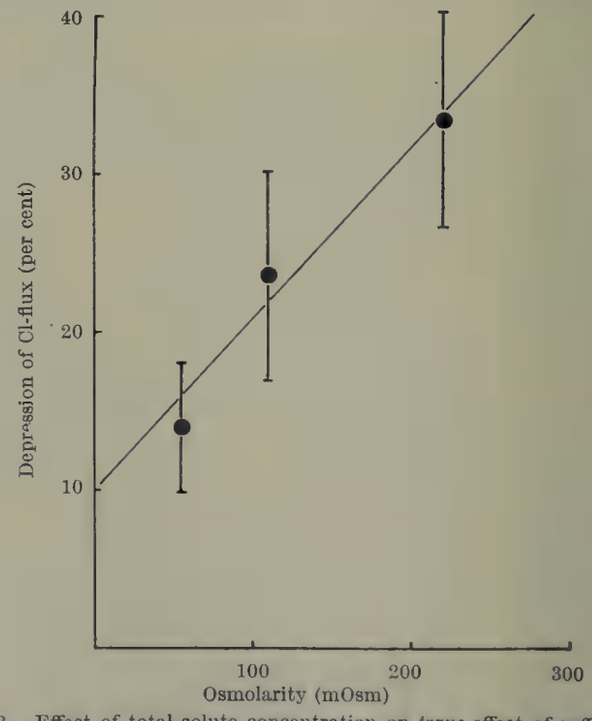

Fig 3. Effect of total solute concentration on *trans* effect of raffinose on the Cl-flux across the short-circuited 'Cellophane' membrane. On the *cis* side, NaCl in osmolar concentrations as indicated. On the *trans* side, 10 mM NaCl and raffinose to complete iso-osmolarity with the *cis* side. Room temperature. Correlation coefficient $r = 0{\cdot}81$ ($P < 0{\cdot}01$)

flow with solute–solvent interaction cannot be the cause of the observed *trans* effect.

A solute–solute interaction, namely between Cl-ions and the *trans* solutes within the membrane pores, seems improbable in view of the large pore radii, which are about 20 Å for dialysing tube and 40 Å for 'Cellophane'[8]. Were it, however, effective, one would expect that the *trans* effect would also occur with non-ionic fluxes, and would increase with the total solute concentration. The *trans* effect on the flux of ^{14}C-urea caused by heavier molecules is almost negligible and may be accounted for by changes in membrane hydration or viscosity. On the other hand, the *trans* effect on Cl-ions does increase if the total solute concentration is raised on both sides of the membrane (Fig. 3).

In summary, the observed *trans* effects cannot be attributed as yet to a single cause. Since they are negligible with non-ionic fluxes, the ionic pattern of the membrane may be important. Although the *trans* effects observed with gastric mucosa are much greater than those with artificial membranes[5], they clearly cannot be exclusively due to a viable transport or exchange mechanism, and to assume free diffusion for passive fluxes across any membrane seems to be a dangerous over-simplification.

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Identification of Dinitro-octylphenols in Certain Commercial Fungicides

A mixture of nitrated alkylphenols is made by various manufacturers in the United States, Great Britain, and The Netherlands, who then esterify it with crotonic or other acid for the preparation of fungicides useful in the control of powdery mildews and also spider mites on various crops. Fractionation of this mixture, hereinafter called DNOP, on alumina (Spence type 'H') in benzene, followed by 2 per cent methanol in benzene, followed by 1 per cent acetic acid in 49 : 1 benzene–methanol, led to the isolation of three eluates[1]. From each of these, amine salts were prepared and analyses showed them all to be of dinitro-octylphenols; the melting points, however, showed them all to differ from each other and also from those of 2,4-dinitro-6-*n*-octylphenol and 2,6-dinitro-4-*n*-octylphenol[2]. The melting points of salts of eluate II, which formed not more than 5 per cent of any of the DNOP samples, were close to those of the corresponding salts of dinocap phenol (2,4-dinitro-6-(1-methylheptyl)phenol)[1,3]. As the biological activities (towards powdery mildews, spider mites, and host plants) of eluates I and II were similar, and those of eluate III were dissimilar, it appeared that eluate I, which formed about 60 per cent of one sample, was likely to be another isomer of dinocap phenol, and eluate III, which formed about 25 per cent of the same sample, an isomer of 2,6-dinitro-4-octylphenol other than the *n*-octyl or 1-methylheptyl[1,3]. The 1-ethylhexyl members of both series (2,4-dinitro-6-octylphenol and 2,6-dinitro-4-octylphenol) have therefore been synthesized, together with the 1-ethylpentyl and 1-ethylheptyl homologues, and the isomeric 2,6-dinitro-4-(1-propylpentyl)phenol. The ethylalkyl compounds were prepared by reaction of the appropriate alkyl magnesium bromide with *o*- or *p*-hydroxypropiophenone to give the *o*- or *p*-1-hydroxyalkylphenol, respectively[4]. Dehydration by refluxing with toluene and a few crystals of iodine under a Dean and Stark tube yielded the alkene, which was reduced catalytically (palladium charcoal) at about 50° C under 500 lb./in.^{2} pressure in 4–6 h to the 1-ethylalkylphenol. Nitration was done in glacial acetic acid, and the dinitro-(1-ethylalkyl)phenols isolated as various amine salts. Salts of the 2,4-dinitro-6-(1-ethylalkyl)phenols were easily recrystallized from benzene or methanol; those of the 2,6-dinitro-compounds required *cyclo*hexene to give a tar-free product economically. The 2,4-dinitrophenols formed suitable salts with a greater range of amines than the 2,6-dinitrophenols. Melting points and analyses are given in Table 1.

Table 1

Phenol	M.p. of amine salt* °C.		Analysis† C Calc.	C Found	H Calc.	H Found	N Calc.	N Found
			%	%	%	%	%	%
2,4-Dinitro-6-(1-ethylpentyl)	M	138-9	55·3	55·3	7·3	7·4	11·4	11·3
	CHA	151	–	–	–	–	–	–
2,4-Dinitro-6-(1-ethylhexyl)	CHA	138	60·7	60·2	8·4	8·2	10·6	11·1
	Pip.	143-4	59·8	60·4	8·1	8·6	11·0	12·3
	M	154-6	56·4	56·4	7·6	7·8	10·9	10·8
	DCHA	153-4	65·4	65·0	9·0	8·7	8·4	8·2
2,6-Dinitro-4-(1-ethylpentyl)	M	150-1	55·3	55·1	7·3	7·7	11·4	11·6
	CHA	173-4	–	–	–	–	–	–
2,6-Dinitro-4-(1-ethylhexyl)‡	CHA	163-5	60·7	60·7	8·4	8·5	10·6	9·9
	M	125	56·4	56·1	7·6	7·6	11·0	11·1
	Pip.	114	–	–	–	–	–	–
2,6-Dinitro-4-(1-ethylheptyl)	CHA	167-8	61·7	61·7	8·6	8·5	10·3	10·5
	M	107-8	–	–	–	–	–	–
2,6-Dinitro-4-(1-propylpentyl)	CHA	181-3	60·7	61·9	8·4	8·6	10·6	10·6
	Pip.	109-11	–	–	–	–	–	–

* CHA = *cyclo*hexylamine; Pip. = piperidine; M = morpholine; DCHA = di*cyclo*hexylamine.
† Analyses by Weiler and Strauss, 164 Banbury Road, Oxford.
‡ Forms salts with DCHA (molten by 60° C) and *N*-ethylpiperidine (m.p. 90° C).

A comparison of the melting-points of the amine salts obtained from DNOP eluates with those of the salts of authentic dinitro-octylphenols (Table 2) led to the conclusion that eluate I was 2,4-dinitro-6-(1-ethylhexyl)-phenol. Four of the five salts of eluate III had melting points agreeing well with those of the corresponding salts of 2,6-dinitro-4-(1-ethylhexyl)phenol, but there was a large discrepancy in the case of the morpholine salts.

Table 2

Phenol	M.p. of amine salt, °C CHA	Pip.	M	DCHA	NEP
Eluate I*	139-41	141-2	150-1	152-3	–
2,4-Dinitro-6-(1-ethylhexyl)	138	143-4	154-6	153-4	–
Eluate II	145-7	107-10	97	–	–
2,4-Dinitro-6-(1-methylheptyl)	151-3	108-10	97-8	149-50	–
Eluate III	160-1	117	161-3	< 60	< 90
2,6-Dinitro-4-(1-methylheptyl)	129-30	144	127	–	–
2,6-Dinitro-4-(1-ethylhexyl)	163-5	114	125	< 60	90

* Eluate I also yielded salts with diamylamine, m.p. 69°–70·5° C, and 2-ethylpiperidine, m.p. 117° C.

To clear up the doubt about the identity of eluate III, nuclear magnetic resonance spectra in carbon tetrachloride of all three eluates, after purification via amine salts, were prepared by Mr. P. J. Ayres, Middlesex Hospital, and the band positions were computed and assigned by Dr. J. A. Elvidge, Imperial College of Science and Technology. Spectra were also prepared of the six dinitro-octylphenols, two *n*-octyl and four *s*-octyl, available at that time. Data obtained are given in τ values (and J c/s) in Tables 3 and 4. The inferences drawn are: (i) in spite of the discrepancy in the melting points of the morpholine salts, eluate III from the DNOP provided by Rohm and Haas Co. is 2,6-dinitro-4-(1-ethylhexyl)phenol; (ii) although the melting points of the piperidine salts of eluate III from May and Baker, Ltd., DNOP and of 2,6-dinitro-4-(1-ethylhexyl)phenol are so similar, the former was a mixture, approximately 1:1, of the latter and the isomer, 2,6-dinitro-4-(1-methylheptyl)phenol (a 1 : 1 mixture of the two

Table 3

2,4-Dinitro-6-(1-ethylhexyl) phenol	Eluate I, purified via piperidine salt	2,4-Dinitro-6-(1-methylheptyl) phenol	2,4-Dinitro-6-*n*-octylphenol	Assignments
9·13 (7*t*)	9·15 (6·5*t*)	9·12 (*ca.*5*t*)	9·11 *ca.t*	ω -Me
9·09 (7·5*d*)	9·08 (6*d*)	8·67 (7*d*)	8·67*s*	1-Me $[CH_2]_5$ $[CH_2]_4$
8·72 *ca.s*	8·73 *ca.s*	8·72 *ca.s*		
8·25*m*	8·26*m*	8·39*m*	8·35*m*	$2\text{-}CH_2+CH_2$ of 1-Et $2\text{-}CH_2$
6·72 (7*q*)	6·75 (6·8*q*)	6·59 (6·8*se*)	7·14 (7·5*t*)	1-CH $1\text{-}CH_2$
2·68*s*	2·69*s*	2·71*s*	2·69*s*	OH
1·65 (2·5*d*)	1·68 (2·5*d*)	1·65 (2·5*d*)	1·66 (2·5*d*)	5H
1·03 (2·5*d*)	1·09 (2·5*d*)	1·10 (2·5*d*)	1·10 (2·5*d*)	3H

Table 4

2,6-Dinitro-4-(1-ethylhexyl) phenol	Eluate III*	Eluate III†	2,6-Dinitro-4-(1-methylheptyl) phenol	2,6-Dinitro-4-*n*-octylphenol	Assignments
9·13 (7*t*)	9·10 (7*t*)	9·12*t*	9·13 (*ca.*5*t*)	9·11 *ca.t*	ω -Me
9·09 (7*d*)	9·08 (6*d*)	9·09*d*	8·69 (6·5*d*)	8·69*s*	1-Me $[CH_2]_5$ $(CH_2)_4$
8·72 *ca.s*	8·74 *ca.s*	8·73 *ca.s*	8·74 *ca.s*		
8·31*m*	8·41*m*	8·35*m*			$2\text{-}CH_2+CH_2$ of 1-Et
			8·33*m*	8·35*m*	$2\text{-}CH_2$
7·41 (7*q*)	7·37*q*	7·26*m*	7·15 (6·8*se*)		1-CH
2·70*s*	2·66*s*	2·64*s*		7.28 (7.5*t*)	$1\text{-}CH_2$
1·91*s*	1·89*s*	1·95*s* 1·88*s*	2·71*s* 1·90*s*	1·87*s*	OH 5-H 3-H

* Purified from Rohm and Haas Co. DNOP via morpholine salt.
† Purified from May and Baker, Ltd., DNOP via piperidine salt.

synthetic salts was found to melt at 118° C, so that there is no discrepancy here); (iii) the identities of eluates I and II, already deduced from the amine salts melting points, are confirmed.

The process used by the various firms manufacturing fungicides based on dinitro-octylphenols therefore produces not only the *para*-substituted phenol (as would be expected) but also causes the alkyl chain to add on to the benzene ring at an unexpected position in the chain, namely, at C_3. It has hitherto been assumed that addition of a chain derived from a 2-hydroxyalkane would take place at C_2.

We thank Mr. P. J. Ayres and Dr. J. A. Elvidge for the provision of evidence by NMR spectra, and May and Baker, Ltd., Murphy Chemical Co., Ltd., n.v. Philips-Duphar, and Rohm and Haas Co. for gifts of DNOP from their respective processes. We also thank Dr. D. Woodcock, Long Ashton Research Station, Bristol, for valuable help in the reduction of the alkenylphenols, and Dr. G. G. S. Dutton for advice on routes of synthesis.

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n-Alkan-1-ols in Butter after Oxidative Deterioration

Chemical mechanisms for the formation and decomposition of hydroperoxides in oxidizing lipids predict the presence of a wide range of compounds. Of these, three classes of aliphatic aldehydes (*n*-alkanals, *n*-alk-2-enals and *n*-alka-2,4-dienals) have long been isolated and identified and more recently the presence of non-conjugated unsaturated aldehydes (hex-*cis*-3-enal[2] and hept-*cis*-4-enal[3]), a vinyl ketone (oct-1-en-3-one[4]) and its corresponding alcohol (oct-1-en-3-ol[5,6]) has also been demonstrated. The *n*-alkan-1-ols, also predicted by the mechanisms, had not hitherto been isolated and identified.

Experiments conducted in these laboratories have now shown that, in addition to oct-1-en-3-ol, *n*-alkan-1-ols are also present in the steam distillate of oxidized butter. Appreciable quantities (less than the *n*-alkanals but greater than the *n*-alk-2-enals) of the $C_{1,2,5-8}$ and minor amounts of the $C_{3,4}$ *n*-alkan-1-ols have been isolated from butter prepared from cream treated with copper and ascorbic acid.

The occurrence of *n*-alkan-1-ols in a degrading lipid system has probably been overlooked as a result of their relative lack of flavour, their difficulty in characterization and the general acceptance that carbonyl compounds are the main contributors to the flavour of oxidized lipid.

Work on the change in ratio of *n*-alkan-1-ols to aldehydes during low-temperature storage of butters will be reported in the *Journal of Dairy Research*.

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Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation–Reduction State of *N*-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate)

It has been previously observed that the rate of photophosphorylation in chromatophores from photosynthetic bacteria was dependent on the oxidation–reduction state of phenazine methosulphate[1]. On addition of ascorbate which reduces phenazine methosulphate non-enzymatically[2], the rate of photophosphorylation was observed to decrease markedly. However, ascorbate has been shown not to have any inhibitory effect on photophosphorylation in isolated chloroplasts catalysed by phenazine methosulphate[3]. Indeed, ascorbate is commonly used to stimulate photophosphorylation with phenazine methosulphate in isolated chloroplasts[3,4].

We have now made a systematic investigation of the effect of the oxidation–reduction state of phenazine methosulphate on photophosphorylation by isolated chloroplasts. The oxidation–reduction state of the system was controlled by using a TPNH-generating system and varying the effective reducing power by changing the concentration of added TPN. The experiments were conducted under strictly anaerobic conditions in the presence of a catalase–ethanol trap to remove traces of oxygen[5].

Fig. 1 illustrates that phenazine methosulphate-catalysed photophosphorylation is indeed dependent on the oxidation–reduction state of the electron carrier. Maximal rates of photophosphorylation were obtained in the presence of 1×10^{-8} M TPN. In the absence of TPN or in the presence of concentrations higher than 10^{-7} M, the rate of photophosphorylation was markedly decreased. Measurement at 388 mμ (ref. 2) showed that the amount of oxidized phenazine methosulphate remaining after the reaction was completed was as follows: 75 per cent in the absence of TPN, 60 per cent with $1{\cdot}3 \times 10^{-8}$ M TPN (optimal) and 0 per cent at and beyond $1{\cdot}3 \times 10^{-6}$ M.

This direct demonstration of the dependence of the rate of photophosphorylation with phenazine methosulphate on the oxidation–reduction state of the system is in agreement with the conclusions of previous investigators using different experimental techniques. Thus, Jagendorf and Avron[6] and Whatley[7] concluded from the effects of *p*-chlorophenyldimethylurea and oxygen that the oxidation–reduction potential of phenazine methosulphate was

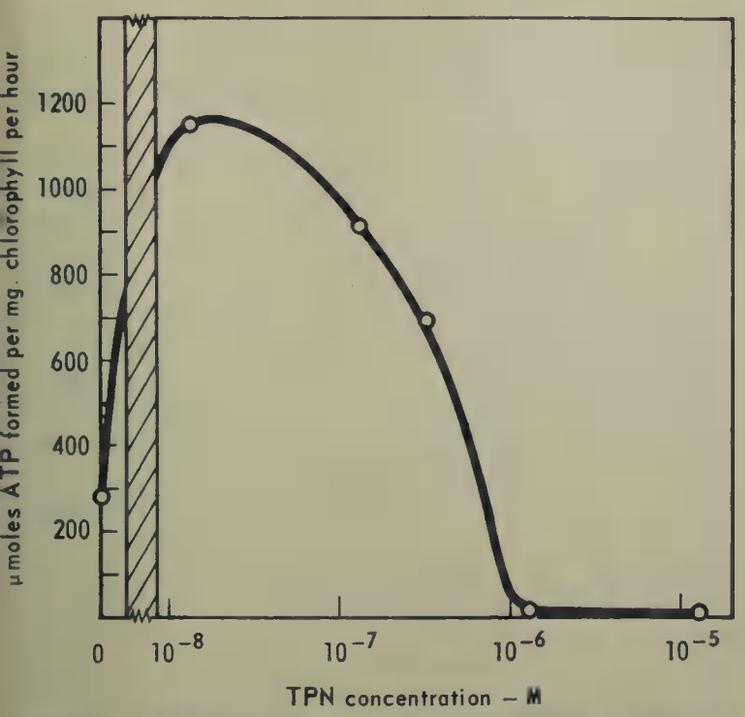

Fig. 1. Photophosphorylation by isolated chloroplasts as affected by TPNH concentration. The reaction mixture included in µmoles or µg: *tris*-HCl, *p*H 7·8, 45; NaCl, 60; $MgCl_2$, 12; Na, K, phosphate, containing 4×10^6 c.p.m. ^{32}P, 12; ADP, *p*H 7·8, 4; phenazine methosulphate, 0·1; ethanol, 700; catalase (Boehringer, crystalline), 50 µg; sodium isocitrate, 10; isocitric dehydrogenase (Sigma, Type IV), 30 µg; TPNH-diaphorase[5] in excess; TPN as indicated; once-washed chloroplasts[4] containing 28 µg of chlorophyll in a final volume of 3·0 ml. The reaction mixture was placed in a modified Thunberg tube fitted with a 1-cm cuvette, evacuated to 25 mm mercury with continuous tapping for 2 min, and closed. Chloroplasts were added from the side arm, and the tubes illuminated at 20° C for 2 min with 160,000 lux of white light filtered through a 'Cellophane' filter transmitting only red light (above 600 mµ). $AT^{32}P$ formed was analysed as previously described[4]

of major importance in determining the rate of photophosphorylation by isolated chloroplasts.

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PHYSIOLOGY

Monocular versus Binocular Visual Acuity

THIS communication describes experiments in which the threshold contrast of gratings viewed monocularly and binocularly have been determined (Fig. 1). We have modified the technique of Schade[1] so that a grating target is generated on an oscilloscope by supplying suitable signals to the *x*, *y* and *z* axes. It could be continuously varied both in contrast and fineness (spatial frequency) without the mean luminance of the screen changing. The energy distribution across the grating varied sinuosoidally. The grating filled a rectangular area subtending 2° by 1·3°, and was surrounded by a circular field of 12° diameter of the same luminance as the oscilloscope screen (80 cd/m²). The screen was viewed from 57 in., and in all the experiments particular care was taken to correct the eye to within 0·12 diopter with spectacle lenses.

To exclude possible artefacts due to fluctuations of accommodation[2] and pupil size, both eyes of one subject (D. G. G.) were fully atropinized. He viewed the screen through artificial pupils (2·8 mm diam.) placed close to the cornea. Special care was taken to centre the pupils[3,4]. The non-viewing eye was covered with a piece of glass sufficiently frosted to obscure the details of the screen while presenting a field of similar luminance to that of the unobscured screen. The subject adjusted the contrast until he was satisfied that the grating could just be resolved. At each spatial frequency, threshold measurements were made with each eye and with both eyes together. The sequence of measurements was made in a random order.

The results are shown in Fig. 2. The general shape and magnitude of the contrast threshold confirm that

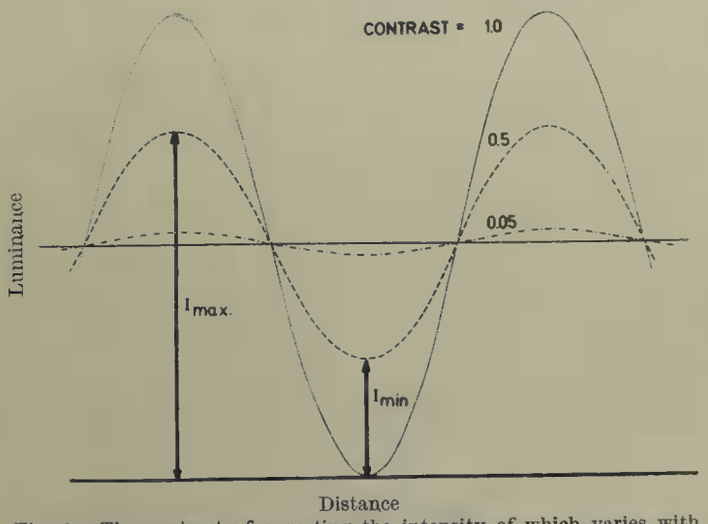

Fig. 1. The contrast of a grating the intensity of which varies with distance sinusoidally is defined as $\frac{I_{max}+I_{min}}{I_{max}-I_{min}}$. Three levels of contrast are shown; 1·0, 0·5 and 0·05. Note that as contrast varies the mean light level across the grating remains constant. The spatial frequency of a grating is the reciprocal of the angular distance between successive maxima in the sinusoidal intensity distribution. Spatial frequency is thus equivalent to visual acuity

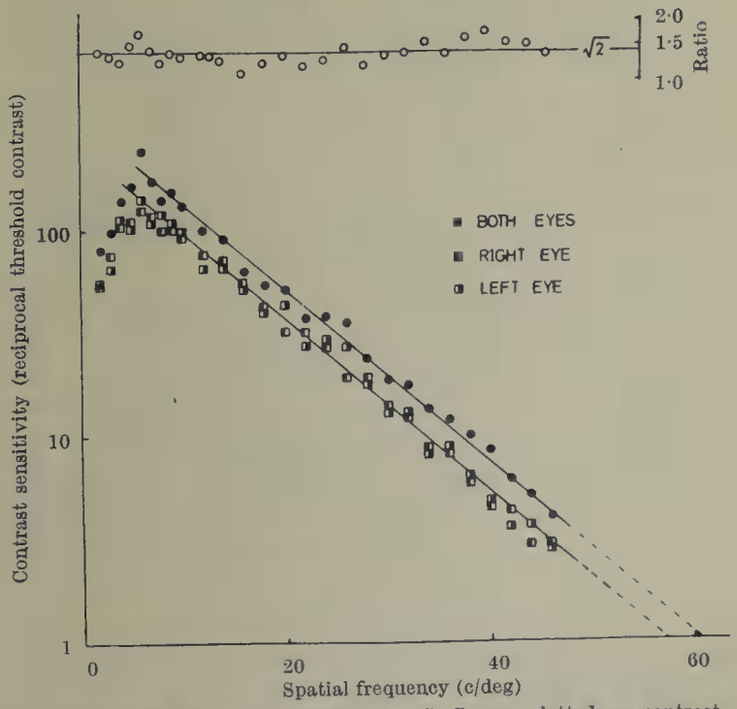

Fig. 2. The results from observer D. G. G. are plotted on a log scale. Contrast sensitivity is defined as the reciprocal of the contrast at threshold. Contrast sensitivity = $\frac{I_{max}-I_{min}}{I_{max}+I_{min}}$. Spatial frequency in c/deg of visual angle is plotted on a linear scale. Each point is an average of two conservations. The two straight lines are placed at a ratio of $\sqrt{2}$ apart in contrast and fitted to the results by eye. The interrupted lines are extrapolations to unit sensitivity (100 per cent contrast). In the upper portion of the figure the open circles represent the ratio, plotted on log scale, of binocular/mean-monocular sensitivity at each spatial frequency. The straight horizontal line corresponds to a ratio of $\sqrt{2}$

found by Schade[1]. It is clear that the threshold contrasts for the left and right eyes are very similar at all spatial frequencies. At each and every frequency the binocular threshold was found to be lower than that of the monocular threshold. That is, binocular sensitivity is greater than monocular.

Plotted along the top of Fig. 2 is the ratio of the binocular to the (arithmetic) mean monocular sensitivity at each observed frequency. A straight line has been drawn corresponding to a ratio of 1·414, that is, $\sqrt{2}$. The mean of the ratios in this experiment is 1·440 ± 0·030 *S.E.* The experiment was repeated on D. G. G., this time using natural pupils and accommodation, and the mean ratio was found to be 1·443 ± 0·032 *S.E.* For subject F. W. C., also with natural pupils and accommodation, the mean ratio obtained was 1·372 ± 0·045 *S.E.* The mean ratio for these three experiments, on two subjects, is 1·418 ± 0·021 *S.E.* None of these ratios differs significantly from $\sqrt{2}$.

The two parallel straight lines drawn through the results for frequencies higher than 6 c/deg are separated by a factor of $\sqrt{2}$ in contrast. The dotted extensions show extrapolations to unity contrast sensitivity; that is, the point where the grating is modulated from zero luminance to a peak level of twice the mean luminance. To avoid non-linearities in the responses of the oscilloscope phosphor, measurements were not made for contrasts higher than 0·4. It is estimated that at unity contrast the threshold frequency for monocular viewing would be 57·0 c/deg and for binocular viewing 61 c/deg. Thus, the calculated improvement in visual acuity for binocular viewing at high contrast would be 61/57·0 = 1·070, that is, 7·0 per cent. Several workers have compared the visual acuity of one eye with two, using high contrast test objects, and have found values ranging from 5 to 10 per cent[5,6]. However, they did not measure the ratio in the contrast domain, where we find improvements of the order of 42 per cent for binocular viewing at all spatial frequencies.

Our findings may be explained if it is assumed that when identical images are fed to two matched eyes their outputs are summed and that these outputs are noisy; that is, contain spurious extra components not correlated in each channel. Because the standard error of the sum of n independent measurements of a random or noisy process decreases as $\sqrt{n}$, an observer using two eyes can obtain two measurements which thus permit a $\sqrt{2}$ lower contrast to be detected.

It is interesting to consider the question of whether covering the other eye with a diffusing screen affects the threshold; the argument being that permitting an equal amount of light, containing no image detail, to reach the obscured eye would lower the contrast sensitivity of the viewing eye by introducing additional noise. To test the effect of the diffusing screen technique, a comparison was made with the effect of an opaque cover, using 30 c/deg as the test frequency for the viewing eye. It was found that the mean ratio of contrast sensitivity for diffuse/obscured presentation to the non-viewing eye was 1·019 ± 0·022 *S.E.* As this mean does not differ significantly from a ratio of 1, it suggests that it makes no difference whether no light, or a diffuse light, enters the non-viewing eye, providing the pupil size of the viewing eye is fixed.

The fact that a stimulus that should have only added noise does not raise the threshold could be interpreted as evidence against a simple hypothesis of signal-to-noise detection. However, it may be that complete addition of signal and noise occurs only when congruent images are presented to the two retinae.

Lythgoe and Phillips[7] and Crozier and Holway[8] compared monocular with binocular luminance thresholds for a patch of light exposed for 0·2 sec; they similarly found that the ratio of the binocular to the average of the monocular thresholds was equal to $\sqrt{2}$. These workers explain their results in terms of Piper's law, which states that the threshold is inversely proportional to the square root of the area of the test field. It is difficult to account for our findings in terms of an area–threshold relationship.

Pirenne[9], who has also measured binocular and monocular absolute thresholds, finds an improvement of a factor of 1·25. As he clearly illustrates, this improvement can be explained by probability summation between independent channels without invoking the physiological summation of signals from the two eyes; a class of argument which must be distinguished from our own which requires the actual physical summation of signals from the two eyes. Recent electrophysiological evidence seems to provide a basis for such physiological summation[10]. Furthermore, a description in terms of the detection of signals in the presence of noise seems to offer a more general explanation[11,12] which can encompass the $\sqrt{2}$ improvement found under such widely differing binocular viewing conditions.

The effect of doubling the signal luminance and presenting it to one eye was next tested. This was done by measuring the contrast sensitivity for identical fields differing only by a factor of 2 in luminance. The field was viewed by D. G. G. through a 2·8 mm-diam. pupil with his atropinized right eye. Ten measurements of threshold were made at each luminance (40 and 80 cd/m²) for a grating of 30 c/deg. The mean ratio of the contrast sensitivity for the higher/lower luminance was found to be 1·173 ± 0·028 *S.E.* This mean ratio differs significantly from 1, and also from 1·440 ± 0·030 *S.E.* found for the same subject viewing the field with both eyes through pupils of 2·8 mm. This result indicates that we are observing at a luminance (80 cd/m²) sufficiently high to be on the 'plateau' where visual acuity is substantially independent of luminance[13]. Thus, at these luminance levels it is advantageous to use a binocular eyepiece for microscopy for contrast sensitivity is improved, even if binocular viewing is achieved by splitting the light into two portions each of half the intensity.

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A Central Action of Hemicholinium

The action of hemicholinium No. 3 (α-α-dimethylethanolamine 4,4′-*bis*acetophenone, HC 3) on the synaptic release of acetylcholine at the neuromuscular junction and within autonomic ganglia is well established[1]. However, apart from the finding that intravenously administered HC 3 reduces the acetylcholine content of the pons and medulla of dogs[2], and the report that locally administered HC 3 reduces the content and synthesis of acetylcholine within the caudate nucleus of rats[3], there

is yet no evidence that this substance affects the output of acetylcholine from central cholinergic synaptic terminals. There is convincing evidence that acetylcholine is the excitatory transmitter released from the axon collateral endings on Renshaw cells of the feline spinal cord[4], and the opportunity was taken to assess the action of HC 3 at these synapses.

The experiments were performed in the lumbar segments of spinal cats anaesthetized with pentobarbital sodium. Renshaw cells were activated synaptically by volleys in the appropriate segmental ventral or dorsal roots, and extracellular spike potentials (negative–positive, 100–1,000 μV in amplitude) were recorded by the sodium chloride-containing centre barrel of five barrel micropipettes[5]. The overall diameter of these micropipettes was 4–6μ, and the other barrels contained aqueous solutions of acetylcholine bromide (1 M); DL-homocysteic acid (sodium salt, 0·2 M, *p*H 7·5); HC 3 bromide (0·1 M); triethylcholine chloride (0·1 M) or sodium chloride (M), from which pharmacologically active ions could be ejected by suitably directed electrophoretic currents[6]. The orthodromically evoked spikes were counted by means of a gated electronic counter, and the responses were also photographed for accurate measurement of latencies.

Control experiments established that although there was some reduction in the number of spikes evoked by prolonged maximal ventral root stimulation at frequencies of 3–10/sec, the number was reasonably stable after the first 5–10 min for a period of at least several hours. In particular there was no appreciable increase in the latency of the first spike. During the electrophoretic administration of HC 3 (5–20 n. amp) there was an increase in the latency of this and subsequent spikes, and often a marked reduction in the total number of spikes. These alterations in firing pattern were first noticed 10–20 min after the beginning of the HC 3 administration and were progressive: in all cases the ventral root was stimulated continuously at the foregoing frequencies. Alterations in the total number of spikes were complicated by a reduction in the sensitivity of some cells to electrophoretically administered acetylcholine, and by actual excitation by the HC 3, as revealed by a facilitation of firing induced by DL-homocysteic acid with or without an actual increase in the 'spontaneous' discharge rate. Excitation by HC 3 was characterized by periodic bursts of high-frequency firing similar to those produced by gallamine and D-tubocurarine[7]; often such a burst followed the delayed first synaptic spike (Fig. 1, *B*, lower record). These effects, presumably due to a direct interaction of HC 3 with postsynaptic structures[1], were usually maximal within a few minutes of the administration of this agent and could be minimized to some extent by using extremely low electrophoretic ejecting currents (less than 10 n. amp).

The most marked effect of HC 3 was an increase in the central latency of the first spike evoked by a supramaximal ventral root stimulus, as illustrated in Fig. 1, and in several instances prolongation of this time beyond 2–4 msec was followed by complete block of synaptic excitation approximately 60–80 min after the beginning of the HC 3 administration. Partial recovery was observed in several instances within 5–10 min of the cessation of the HC 3 administration (Fig. 1, *D*). As dihydro-β-erythroidine, a powerful antagonist of the nicotinic receptors of Renshaw cells[4], has little or no effect on the latency of the first spike evoked by such a stimulus, it is unlikely that the increase of latency produced by HC 3 is the result of a postsynaptic action, particularly as increases were observed when the actual sensitivity of Renshaw cells to acetylcholine was undiminished. Hence it may be reasonably proposed that in the presence of HC 3 the output of acetylcholine per stimulus becomes diminished, with a consequent reduction in the rate of rise of the resultant excitatory postsynaptic potential. Two factors may be involved in the time over which it was necessary to stimulate the ventral root repetitively before such a presynaptic action could be demonstrated: the presynaptic store of acetylcholine is evidently comparatively large compared with the amount released per impulse, and a maximum ventral root stimulus normally results in the release of a large amount of acetylcholine in the environment of a particular cell compared with the amount necessary to produce an above-threshold postsynaptic depolarization.

Renshaw cells may also be fired synaptically by dorsal root volleys without the involvement of motoneurones[7]. Not all cells responded to such afferent excitation, and many did not follow repetitive stimulation (3–10/sec) of the dorsal root, presumably because of the polysynaptic nature of the excitatory pathway. However, in one case, conditions were satisfactory and the responses of this cell to dorsal root stimuli at a frequency of 3·3/sec were unaltered after 60 min administration of HC 3, at which time the latency of the first ventral root-induced spike had been increased from less than 1 msec to approximately 2·8 msec. This finding confirms other pharmacological evidence that the firing of Renshaw cells by dorsal root afferent volleys may not involve cholinergic mechanisms[7], and also suggests that the presynaptic action of HC 3 may be specific for cholinergic terminals. Additional evidence for this latter proposal was the finding that prolonged administration of HC 3 failed to influence the synaptic excitation of dorsal horn interneurones, which are presumably not innervated by cholinergic fibres[7].

Experiments were also carried out on Renshaw cells using triethyl(2-hydroxyethyl)ammonium (triethylcholine); but the use of this agent was complicated by a marked depressant action on the sensitivity of the cells to acetylcholine, and a strong excitant action similar to that observed with HC 3. Attempts were made to influence the synaptic firing of Renshaw cells by inhibition of choline acetylase, using electrophoretically administered fluoracetate, *p*-chloromercuribenzoate or diphenylylethylacetic acid[8]. These experiments were unsuccessful, possibly because of the failure to obtain a sufficiently high concentration of the inhibitors at the site of acetylcholine synthesis. Intravenously administered HC 3 (10 mg/kg) was also ineffective, probably for the same reason.

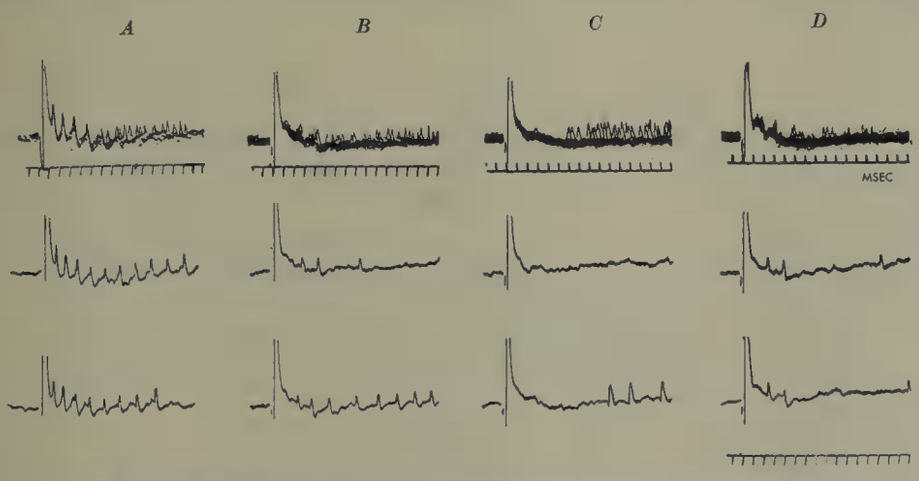

Fig. 1. Spike responses of a single Renshaw cell (upper records—approximately 20 superimposed tracings at a frequency of 5/sec; lower records—single tracings); (*A*) before, (*B*) at 31 min and (*C*) 61 min during, and (*D*) 3 min after the electrophoretic ejection of HC 3 for 69 min (10 n. amp for 59 min; 40 n. amp for 10 min). The measured latencies of the first spike are (*A*) 1·21 msec; (*B*) 2·42 msec; (*C*) 5·8 msec and (*D*) 1·9 msec from the ventral root stimulus. In many of the records of (*C*) the neurone failed to respond to ventral root stimulation and the latency was measured from the superimposed tracing. The sensitivity of this cell to acetylcholine and DL-homocysteic acid was enhanced by HC 3. The ventral root was stimulated supramaximally at a frequency of 5/sec throughout the series, and had been so stimulated continuously for approximately 1 h beforehand. Time, msec for all records. The spikes were approximately 300 μV in amplitude

In conclusion HC 3, which presumably prevents the transfer of choline into central presynaptic cholinergic terminals, thus limiting the rate of acetylcholine synthesis[1], is suitable for testing the cholinergic nature of central synapses, provided the appropriate pathway is activated sufficiently to exhaust the transmitter stored within the endings.

We thank Prof. R. Paoletti for a supply of diphenylylethylacetic acid.

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HAEMATOLOGY

Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase

Within the group of non-spherocytic haemolytic anaemias classified as 'type 11' by Selwyn and Dacie[1], Valentine, Tanaka and Miwa[2] found a specific erythrocyte deficiency of pyruvate kinase (PK). The leucocyte PK activity in patients with this disease is normal[2], suggesting that the genetic control of leucocyte PK differs from that of red cell PK. Electrophoretic differences among PK preparations from rat kidney, liver, cardiac muscle, brain and skeletal muscle were reported by Fellenberg *et al.*[3]. Chromatographic, electrophoretic, solubility and structural differences between the normal human leucocyte and red cell PK have been presented[4].

Pyruvate kinase catalyses the conversion of phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP. Boyer[5] has reviewed studies of enzyme kinetics for rabbit muscle PK. He concludes that phosphoryl transfer occurs by a direct transfer of the phosphoryl group from the donor to the acceptor substrate; that there is one binding site on PK for ATP and ADP and another site for pyruvate or PEP; and that in the forward reaction non-compulsory or independent combination with ADP or PEP occurs with 'equilibrium kinetics' applying. This report describes an investigation of the kinetics of human red cell and leucocyte PK purified by methods previously described[4]. We observed properties for the human leucocyte PK identical to those described for rabbit muscle enzyme. The Michaelis constant (Fig. 1) observed for each substrate is independent of the concentration of the second substrate. The K_m values for PEP of $1{\cdot}0 \times 10^{-4}$ M, and for ADP of $1{\cdot}78 \times 10^{-4}$ M, are very similar to the values reported[6] for the rabbit muscle preparation. The relation between the general kinetic coefficients, $\frac{\varphi_1 \times \varphi_2}{\varphi_{12}} = \varphi_0$ according to Dalziel[7], is compatible with a mechanism of random addition of substrates in which the combination of the enzyme with one substrate does not influence its affinity for the second substrate.

The red cell enzyme has different kinetic properties. At least ten-fold higher concentrations of PEP are required, and apparent Michaelis constants for each substrate vary with the concentration of the second substrate (Fig. 2). Initial velocities measured in the presence of one of the products, ATP, obey the general equation:

$$\frac{(E_0)}{v} = \varphi_0 + \frac{\varphi_1}{(\mathrm{PEP})}\left[1 + \frac{(\mathrm{ATP})}{K_{\mathrm{ATP}}}\right] + \frac{\varphi_2}{(\mathrm{ADP})} + \frac{\varphi_{12}}{(\mathrm{PEP})\,(\mathrm{ADP})}\left[1 + \frac{(\mathrm{ATP})}{K_{\mathrm{ATP}}}\right]$$

These findings suggest competitive inhibition of ATP for PEP with an inhibition constant, K_{ATP}, of $3{\cdot}5 \times 10^{-4}$ M. Only an ordered binding of substrates will account for these results. Because of the assay system used, we have not yet studied the reverse reaction, nor the effect of pyruvate on initial velocities of the forward reaction. For these reasons, we cannot be more specific about the mechanism for the red cell enzyme[8].

The demonstrated differences in chemical[4] and kinetic properties of erythrocyte and leucocyte PK support the known difference in genetic control[2] of this enzyme. Evaluation of kinetic differences between normal and mutant erythrocyte enzyme, however, must take into account the more complicated mechanism of action of erythrocyte PK. Waller and Lohr[9] have reported a higher Michaelis constant of red cell PK for PEP in patients with pyruvate kinase deficiency. Three unrelated patients in whom we have studied the kinetics of purified red cell enzyme showed no difference from normal for both sub-

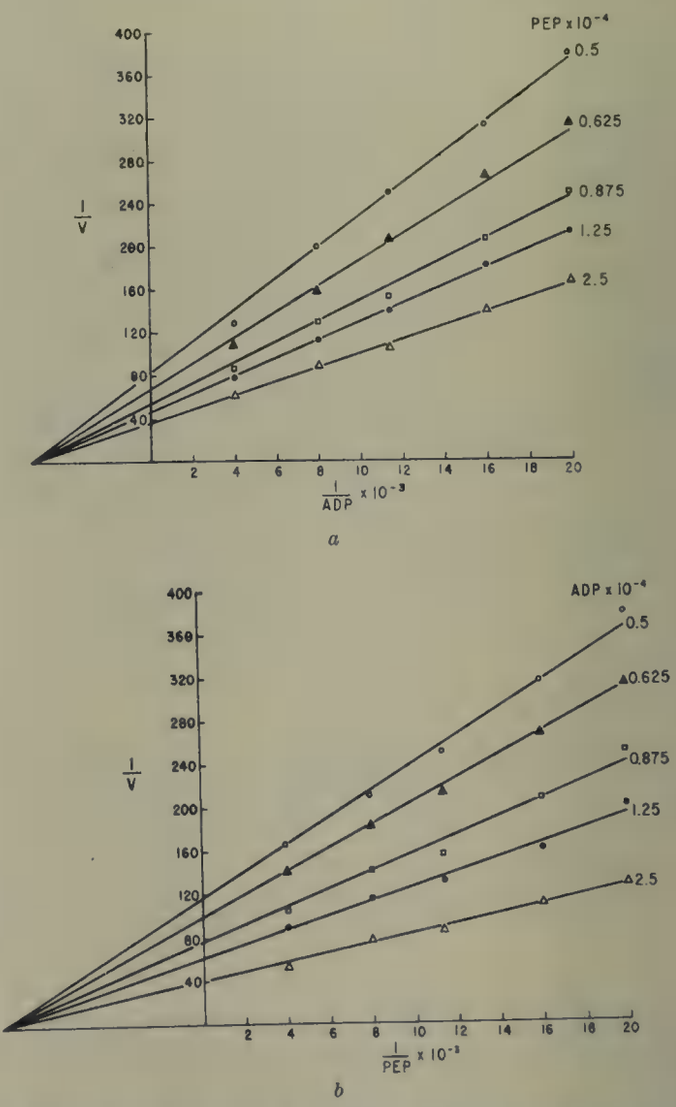

Fig. 1. (*a*) Effect of ADP on the velocity of the leucocyte PK reaction at various PEP concentrations. Assays were made at 37° C in a total volume of 3·0 ml. with 0·075 M K+; 0·016 M Mg++; 0·008 M triethanolamine HCl at *p*H 7·4, and PEP at 0·5; 0·625; 0·875; 1·25 and $2{\cdot}5 \times 10^{-4}$ M. The initial velocity is in micromoles of DPNH oxidized per min (method of Bücher and Pfleiderer[10]). Intersection at the negative abscissa gives an estimate for K_{ADP} of $1{\cdot}78 \times 10^{-4}$ M. (*b*) Effect of PEP on the velocity of the leucocyte PK reaction at 0·5; 0·625; 0·875; 1·25 and $2{\cdot}5 \times 10^{-4}$ M ADP concentrations. Conditions were the same as above. Estimate for K_{PEP} of 1×10^{-4} M

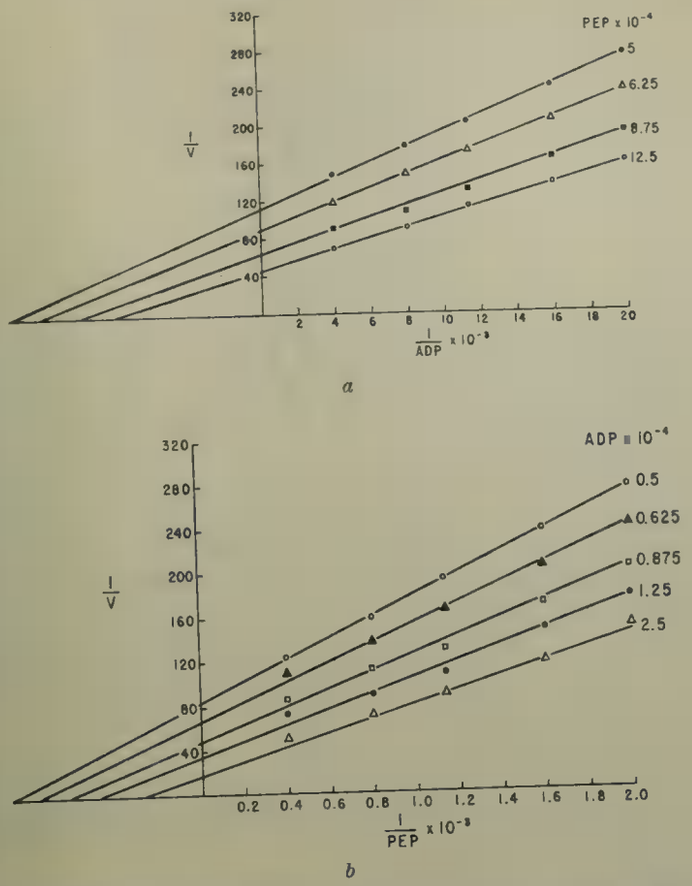

Fig. 2. (*a*) Effect of ADP on the velocity of the red cell PK reaction at 5; 6·25; 8·75 and 12·5 × 10⁻⁴ M PEP concentrations. Conditions were the same as in Fig. 1. (*b*) Effect of PEP on the velocity of the red cell PK reaction at 0·5; 0·625; 0·875; 1·25 and 2·5 × 10⁻⁴ M ADP concentrations. Conditions were the same as in Fig. 1

strates. This would suggest a control gene mutation involved in this disease.

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Inhibition of Haemolysis by 'Cremophor' in Conserved Blood

The biological value of conserved blood is determined by the erythrocyte survival time after re-infusion which, after storage for 3–4 weeks, is so low as to prohibit further use of the erythrocytes. The plasma, however, may be used for several other purposes if it is not too haemolytic. After a lapse of about the same time the haemolysis rate increases remarkably so that blood banks are often obliged to discard whole-blood conserves after the date of expiration.

Claims have been made that vitamin E possesses haemolysis-inhibiting properties in blood banks[1–5]. In an attempt to verify this quality of vitamin E and to elucidate its mechanism of action we added solutions of tocopherol acetate (50 mg/ml.) in a ratio of 2 per cent v/v to ACD-blood. As an emulgator of the water-insoluble vitamin E, these solutions contained 'Cremophor *EL*'* (manufactured by Badische Anilin- u. Sodafabrik AG., Ludwigshafen) in concentrations varying between 40 and 200 mg/ml. Appropriate controls were run with 'Cremophor *EL*' only. These banks were judged visually as to haemolysis after a storage time of 40–80 days. Haemoglobin determinations were carried out in the supernatant ACD-plasma after centrifugation. The results showed, much to our surprise, that 'Cremophor *EL*' alone has a haemolysis-inhibiting effect far exceeding a rather questionable effect of vitamin E. The plasma of ACD-blood to which solutions of 40–200 mg/ml. 'Cremophor *EL*' had been added (final concentration 0·8–4 mg/ml.) remained perfectly clear after a storage time of 40–80 days and contained no appreciable amounts of haemoglobin, whereas the ACD controls were severely haemolytic. Indeed, the addition of vitamin E in low concentration ranges of 'Cremophor *EL*' seemed to block even the 'Cremophor *EL*' action. It seems possible that the surface activity of 'Cremophor *EL*', exhausted by vitamin E in the investigations with low concentrations of 'Cremophor *EL*', represents the key to the mode of action. Investigation of the activity of other surface-active agents seems therefore indicated. From the technical point of view the haemolysis of ACD-blood can be virtually abolished by addition of 'Cremophor *EL*', up to a storage time of about 80 days (perhaps even longer).

It remains an open question whether, in the papers cited here, surface-active agents have interfered, as few data concerning controls with such substances are mentioned. However, from the data given in refs. 2, 3 and 5 it can be concluded that 'Cremophor *EL*' has been used as an emulgator.

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RADIOBIOLOGY

Uptake by Plants of Radiostrontium from Contaminated Soils

In a recent report from this department[1] it was shown that the extractability of radiostrontium from contaminated soil samples was effectively reduced by heat treatment and by the addition of phosphate to the soil. It was pointed out that, under emergency conditions, heat-treatment of the contaminated soil surface and heavy phosphate application might thus reduce the uptake by plants of radiostrontium more efficiently than liming, which is only effective in soils of low calcium status. In the investigation reviewed here the influence of heat treatment and superphosphate application on the plant uptake of radiostrontium was examined in pot experiments. For comparison the effect of applying calcium carbonate to the contaminated soil surface was also determined.

Spring barley (variety 'Pallas') was grown to maturity in PVC pots, each containing 22 kg of soil. Two loamy and

Table 1. Analysis of Soil

Locality	Soil type	pH in water	Cation-exchange capacity (m. equiv./100 g)	Exchangeable Ca^{++} (m. equiv./100 g)	Ca-saturation (%)	Organic matter (%)
Blangstedgaard	Loamy	7·6	15·2	12·6	83	2·2
Ødum	Loamy	7·0	9·2	6·1	66	1·6
St. Jyndevad	Sandy	6·5	11·1	3·9	35	2·4
Borris	Sandy	6·4	10·5	3·7	35	1·9

two sandy soils were used (Table 1). 11 kg of soil was put into each pot, and 40 μc. of carrier-free ^{89}Sr in 20 ml. of water was sprayed evenly over the surface before the superphosphate and calcium carbonate were added. For the heat treatment the contamination was performed by adding the carrier-free ^{89}Sr to 160-g samples of soil, which were then air-dried and heated to the desired temperature for 10 min. The contaminated sample was evenly distributed over the surface of the 11 kg of soil in the pot. The additional 11 kg was then added, and the seeds were sown. It was attempted with this experimental technique to imitate a surface contamination followed by treatments and ploughing. The following treatments were examined:

Control : 40 μc. ^{89}Sr per pot.
T–500 : 40 μc. ^{89}Sr and heating to 500° C.
T–800 : 40 μc. ^{89}Sr and heating to 800° C.
P–1 : 40 μc. ^{89}Sr + 10 g superphosphate (equiv. to 2 tons/ha).
P–2 : 40 μc. ^{89}Sr + 20 g superphosphate (4 tons/ha).
Ca–P : 40 μc. ^{89}Sr + 75 g $CaCO_3$ + 20 g superphosphate (15 and 4 tons/ha resp.).
Ca–1 : 40 μc. ^{89}Sr + 37·5 g $CaCO_3$ (7·5 tons/ha).
Ca–2 : 40 μc. ^{89}Sr + 75 g $CaCO_3$ (15 tons/ha).

The concentration of ^{89}Sr in grain and straw is given in Table 2.

Table 2. The Concentration of Strontium-89 in Barley grown in Four Contaminated Soil Types in Pot Experiments

mμc. ^{89}Sr/g dry matter in barley grown in soils from:

Treatment	Blangstedgaard Grain	Blangstedgaard Straw	Ødum Grain	Ødum Straw	St. Jyndevad Grain	St. Jyndevad Straw	Borris Grain	Borris Straw
Control	0·48	3·63	0·74	4·64	0·67	4·75	1·12	6·89
T–500	0·29	1·99	0·45	3·00	0·50	3·87	0·89	4·73
T–800	0·020	0·15	0·036	0·21	0·21	1·23	0·26	1·27
P–1	0·25	1·82	0·41	2·81	0·27	1·83	0·39	2·33
P–2	0·14	1·16	0·24	1·85	0·16	1·13	0·26	1·45
Ca–P	0·29	2·32	0·47	2·90	0·40	2·42	0·63	3·57
Ca–1	0·44	3·65	0·82	4·95	0·67	4·66	1·36	7·17
Ca–2	0·52	3·44	0·69	4·98	0·72	4·84	1·42	7·53

Heating the contaminated soil to 800° C reduced the uptake of radiostrontium by plants from loamy soils by a factor of twenty. In the sandy soils only a four-fold decrease in the uptake was found. The difference between loamy and sandy soils is greater than expected from the extractability investigations[1]; but this might be ascribed to differences in the mineral composition of the soils used. The fixation of radiostrontium is probably caused by an alteration of the clay minerals at high temperatures. The results indicate that a very effective reduction in the uptake from loamy soils can be obtained by heating the contaminated soil surface; but the practical application of this method is complicated by the high temperatures needed.

Addition of superphosphate caused a marked reduction in the plant uptake of radiostrontium. The largest amount —equivalent to 4,000 kg per hectare—resulted in a four-fold decrease in the strontium-89 uptake from the sandy soils; in the loamy soils the reduction factor was three. It is known from fertilizer experiments that the plants utilize only a small part of the phosphorus added in superphosphate. The remaining portion is converted into phosphate compounds of lower solubility, for example, dicalcium phosphate, octacalcium phosphate and hydroxyapatites. It seems reasonable to expect that a co-precipitation of slightly soluble calcium–strontium phosphate would occur in contaminated soils if sufficient amounts of phosphate were added. However, most of the previous experiments on the effects of phosphate application to contaminated soils[2–4] have shown only a small reduction in the plant uptake of radiostrontium. Although Uhler[5] used massive amounts of ammonium phosphate (phosphorus content equivalent to that in 28·5 tons of superphosphate/ha), the uptake from a calcareous soil was only reduced by 50 per cent, and no effect was seen on an acid soil. The radiostrontium and phosphates were usually mixed into the entire rooting medium, and consequently the phosphate concentration may have been too low to cause precipitation. In the present investigation a high concentration of phosphate in close contact with the strontium-89 was assured by applying the superphosphate directly to the contaminated soil surface. The results obtained by means of this technique could be interpreted as increased precipitation of strontium in the form of slightly soluble phosphates, and thus a reduced plant availability. Addition of calcium carbonate together with the superphosphate decreased the effect as compared with superphosphate alone.

Addition of calcium carbonate did not reduce the uptake of radiostrontium from these soils. On the contrary, the results indicate a slight increase in the uptake, even from the sandy soils of relatively low calcium saturation. The high concentration of calcium carbonate in intimate contact with the contaminated soil layer has apparently caused an increased plant availability of the radiostrontium. This is in agreement with the solubility characteristics reported by Schroeder *et al.*[6] and the suggestion of Wiklander[7] that heavy application of lime might lead to increased solubility of strontium.

Preliminary results from a similar—but long-term—experiment with the same four soils in microplots in the field show the same tendencies as the results of the pot experiment reported here.

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Mechanism of Uptake of Trace Elements by Plant Roots

It is well known that chemical elements can be concentrated in very large factors by plant cells, that is to say, the final concentrations in the cells may exceed those in the media by many orders of magnitude. Enrichment is often observed not only for elements that are essential for the plants, but also for elements not known to be essential, and even sometimes for elements that normally do not occur to any extent in plants.

The question thus arises as to what mechanism is responsible for the elements being taken up against concentration gradients. In the case of some major nutrients, accumulation is due to 'active transport', that is, the required osmotic work is done at the expense of metabolic free energy. For example, much of the uptake of potassium is due either to its own active transport or to a potential difference set up in the active transport of another ion[1]. However, so far as we are aware the importance of active transport does not appear to have been demonstrated for the accumulation of 'trace elements', here to be defined as elements needed by plants only in minute quantities, or not needed at all.

The nature of the mechanism responsible for the accumulation of trace elements is of basic importance to agriculture and also to the evaluation of hazards due to

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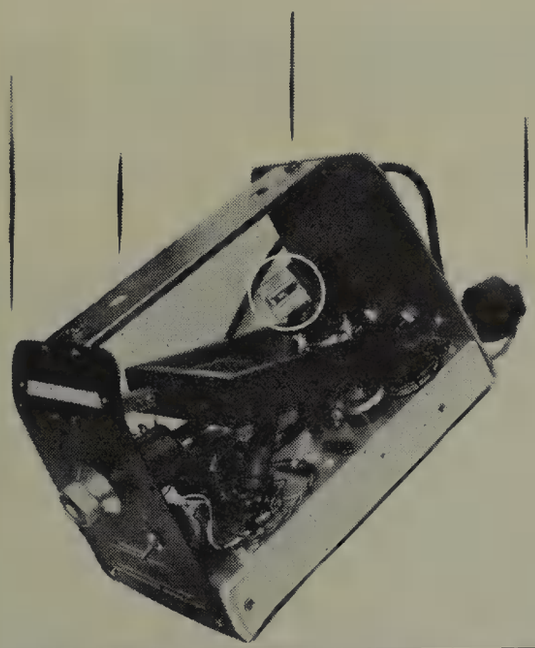

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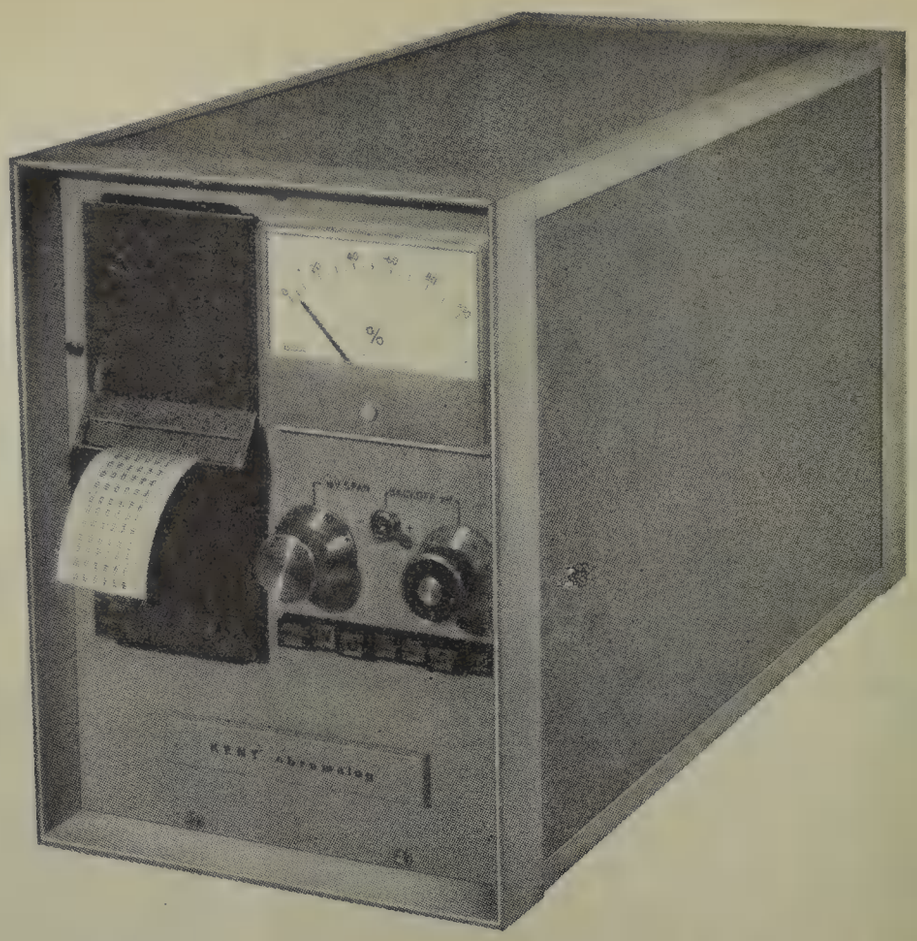

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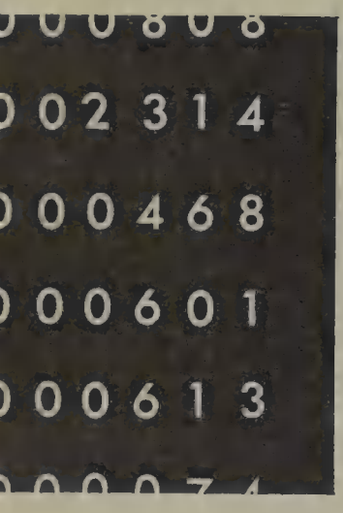

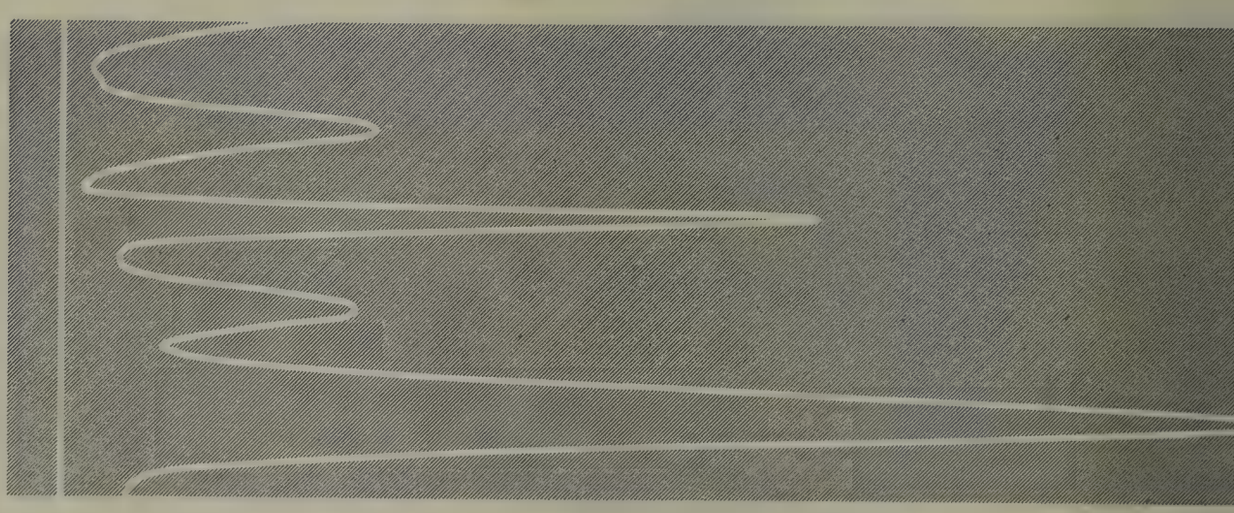

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radionuclides, especially from nuclear explosions (fall-out) or from nuclear reactors. Therefore, following up preliminary experiments with unicellular algae (*Chlorella*)[2], we have studied the uptake of zinc, an essential element, by roots of a crop plant.

We used barley plants that had been grown for about 10 days in tap water. The roots were about 10 cm long; they were cut from the plants 4 h before the start of the experiments and washed several times with distilled water. Normally 1–2 g of roots were shaken with 10 ml. of a solution of radiozinc (zinc-65; half-life 245 days), and thereby aerated, for a few hours. The uptake was computed from the remaining activity of the solutions, as measured with a Geiger liquid counter after removal of the roots. For comparison, analogous experiments were performed using radiopotassium (potassium-42; half-life 12·5 h). In a few experiments, longer-lived rubidium-86 (18·7 days) was substituted for potassium; it is well known that rubidium and potassium behave similarly in respect to uptake[3]. Occasionally, experiments were repeated without severing the roots from the green parts, but the results were not very different. The enrichment factor (defined as the ratio of the concentration in tissue (roots) to that in the supernatant) for zinc was high—about 10^2 in the case of zinc concentrations of the order 10^{-7} M in the supernatant—and depended on experimental conditions. However, the mechanism of enrichment is clearly quite different from that for potassium.

(1) Equilibrium of zinc between roots and solution was reached within a few minutes while uptake of potassium continued for much longer periods. (2) The 'decoupling' metabolic poison dinitrophenol (DNP), in concentrations up to 10^{-2} molar (M), had no influence on the uptake of zinc, while the uptake of potassium was depressed to less than 10 per cent of the normal value; similar results were also obtained with sodium azide. (3) After the roots had been killed by treatment with alcohol for 10 min, the uptake of potassium was practically zero, while that of zinc increased by 50–100 per cent. (4) After root tissue had been homogenized by grinding with glass, the uptake of potassium was almost completely suppressed, but that of zinc again increased two-fold. (5) While the uptake of potassium was not influenced by most foreign ions, for example, sodium or magnesium, in moderate concentrations (up to 10^{-2} M), foreign ions generally competed strongly with zinc: for example, the elements lanthanum, nickel, calcium, magnesium, cobalt, manganese, strontium and barium displaced radiozinc with efficiencies decreasing in that order, namely 138–35 per cent as strongly as inactive zinc of equal (2×10^{-4}) molarity. (6) At least in the range 0°–20° C, and during a period of 5 h, the temperature coefficient for the uptake of radiozinc by intact roots was very much (about a factor of 10) smaller than that for the uptake of radiorubidium.

We suggest that the accumulation of zinc by the plants is due to passive processes, not dependent on metabolism or the integrity of the cells, namely, to exchange adsorption and compound formation[4]. Processes not linked with metabolism are, of course, also observed with potassium[3,5], but in experiments over longer periods active processes predominate.

We thank the Bundeskanzleramt-Verstaatlichte Unternehmungen (Section IV), Republic of Austria, for financial support.

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BIOLOGY

Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes

It has been demonstrated experimentally that brood patch formation in certain passerine birds (in which typically, only the females develop brood patches and incubate in Nature) depends on oestrogen and prolactin action[1].

In the phalaropes (a sub-family of three species only, two holarctic, one Wilson's (*Steganopus tricolor*), nearctic) only males incubate and only they form brood patches. Johns and Pfeiffer[2] showed experimentally that the hormonal stimuli for brood patch formation in two species of phalaropes (one of which was Wilson's) are an androgen and prolactin.

One of us has made a preliminary publication[3] citing data on the gonadal concentrations of the principal sex hormones of breeding Wilson's phalaropes in comparison with those of some other birds. He does not now altogether accept the interpretation given[3] to these data, but the results reported show that female phalaropes are not deficient but rather 'high' in gonadal androgens and hence are unlikely to be poor androgen secretors. Therefore their failure to form brood patches might depend on a deficiency of prolactin, rather than a lack of androgen production.

This communication reports the results of experiments with: implants of the pituitaries of Wilson's phalaropes or Killdeer plovers (*Charadrius vociferus*) on the crop sac of common domestic pigeons by the technique described elsewhere[1]; or with intradermal injections of pituitary extract-suspensions over the crop sac, followed in both cases by the technique of examination previously outlined by one of us[4]. Table 1 summarizes the results obtained. Although in no case did we obtain a full-blown crop sac development with up to 10γ of prolactin (ovine supplied by the Endocrinology Study Section, U.S. National Institutes of Health) or with our avian materials, the data show that breeding male phalaropes produce some prolactin and females significantly less or none. Our two tests on Killdeer material permit no conclusion, but they are compatible with the view that since both sexes of this bird develop brood patches both may form prolactin.

Supporting evidence comes from the data on relative pituitary weight of breeding season Wilson's phalaropes, red-winged blackbirds (*Agelaius phoeniceus*) and of Killdeers which are summarized in Table 2. It should be noted that one of us has previously demonstrated the presence of prolactin in female (males were not tested) red-winged blackbird pituitaries[5].

We can see no obvious *a priori* reasons for sexual differences in adenohypophyseal function in breeding birds of the three representative species examined, other than relative differences in prolactin production. We believe

Table 1. Pigeon Crop Sac Tests for Prolactin, using Implants (*I*) or Intradermal Injections of Saline or Water Extract Suspensions (*E*) of Bird Pituitaries applied to the Crop Sac for 4 Days

Material applied to experimental side	Response	Material applied to control side	Response
15 *M* phal. pits. *I*	− +	Piece of phal. brain	0
14 *M* phal. pits. *I*	− +	Piece of phal. brain	0
39 *M* phal. pits. *E*	+	Prolactin 10γ	+
20 *M* phal. pits. *E*	0	Prolactin 10γ	+
17 *M* phal. pits. + 3 doses *E* of *M*. phal. pits.	+	Nil	0
18 *F* phal. pits. *I*	0	Piece of phal. brain	0
40 *F* phal. pits. *E**	0	Prolactin 3γ	− +
15 *M* Killdeer pits. *E*	− +	Prolactin 10γ	− +
15 *F* Killdeer pits. *E*	0	Prolactin 10γ	− +

* Pigeon died on third day of treatment.
Responses were evaluated by macroscopic and microscopic examination of the crop sacs and rated as follows: 0, no response; − +, suggestion of a positive response; +, minimal but definite positive response.
Abbreviations: *M*, male; *F*, female; phalarope pituitaries, Wilson's phalarope pituitaries.

Table 2. Relative Pituitary Weight (Pituitary Weight in mg per 50 g Body-weight; the Approximate Mean Body-weight of Male Wilson's Phalaropes) in Both Sexes of Breeding Wilson's Phalaropes and Two Other Species of Birds

Female Wilson's phalarope (28)	1·20 ± 0·04
Male Wilson's phalarope (60)*	1·50 ± 0·11
Female red-winged blackbird (22)*	1·81 ± 0·12
Male red-winged blackbird (20)	1·26 ± 0·16
Female Killdeer plover (29)*	0·86 ± 0·07
Male Killdeer plover (36)*	0·86 ± 0·07

Figures in parentheses indicate the number of birds examined.
An asterisk denotes the sex which naturally develops a brood patch and incubates.

that, in birds in general, prolactin is the hormonal stimulus to incubation and brood patch development[6], except in pigeons and doves where, since it controls crop sac development in members of this family, a special case is involved (see refs. 7 and 8).

It is evident from the findings given in Table 2 that in the first two species, in which only one sex naturally develops a brood patch and incubates, that sex has the higher relative pituitary weight. In the Killdeer, in which both sexes form brood patches and incubate, relative pituitary weights are similar. The unit of body-weight (50 gm), relative to which we have expressed pituitary weights in Table 2, is arbitrary but approximates the mean body-weight of the smaller of the two sexes in the first two species.

Further evidence in favour of greater prolactin production in male than in female phalaropes was obtained by a comparison of the number of acidophils per unit area of adenohypophysis in microscopic sections of this tissue from 3 males and 3 female red-necked phalaropes (*Phalaropus lobatus*), all in breeding condition. Acidophils were stained with acid fuchsin by a method previously described[9]. Mean numbers of acidophils per unit standard area (based on counts over 8 such areas for each bird, representing about one-fifth of the total area of the adenohypophysis cut at 5μ) were: males 236 ± 16·0 and females 125 ± 41·5. A paired *t* test applied to the data gave a *P* value for this difference of the means of about 0·08.

There is good evidence that prolactin is produced by fuchsinophilic acidophils in birds[10,11].

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Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents

A collection of 55 published records of population densities of rodents of the genus *Microtus* reveals a strong correlation between levels reached at population peaks and local soil sodium-levels as reported in soil surveys or deduced from indirect evidence. In areas with low soil sodium the highest density reported was 230 animals per acre and populations exceeded 30 individuals per acre in only 12 (19 per cent) of 63 reports. In regions of intermediate soil sodium, *Microtus* populations ranged up to 400 with 16 of 22 (72 per cent) reports listing more than 30 per acre. In regions of high soil sodium there were 14 records in excess of 1,000 animals per acre and 30 of 33 (91 per cent) records were more than 30. Cyclic population fluctuations seem to occur in the animals regardless of sodium-levels, but the densities reached at population peaks are characteristically many times higher in regions of high soil sodium than in regions of low soil sodium. The correlation could be due to other ions associated with sodium in the soil. However, the possibility that it reflects a functional relationship between availability of sodium and population regulation suggested a series of laboratory experiments with meadow voles (*Microtus pennsylvanicus*) from a laboratory colony established with wild animals trapped at Madison, Wisconsin.

Two experiments were conducted to test the effect of restricted and unrestricted sodium chloride diets on reproduction and population growth. Eight 3′ × 6′ pens, each containing two males and two females, and eight 3′ × 3′ pens, each containing one male and three females, were established and left undisturbed for 15 and 19 weeks, respectively. Alfalfa hay and whole oats were supplied in excess to all animals. Four replicates of each sex-ratio group were supplied *ad lib.* with a 0·5 per cent NaCl solution and with distilled water; the control groups received only distilled water. Populations supplied with the NaCl solution produced 60 young in the 2 male : 2 female groups and 104 in the 1 male : 3 female groups compared to 43 and 64 in the restricted sodium diet groups (*P* less than 0·05, paired *t*-test). The groups with sodium *ad lib.* maintained a higher net population over the test periods (*P* less than 0·05, paired *t*-test).

In a second group of experiments segregated sex and mixed sex groups of 5- to 8-week-old voles were tested for self-selection of NaCl at various levels of crowding. They were provided with a choice of distilled water and NaCl solution in paired 'j' tubes, which were calibrated for direct measurement of daily fluid consumption. Three series of tests were conducted: (*a*) high-density groups (16 animals per pen) and low-density groups (4 animals per pen) of each sex were compared directly; (*b*) 24 animals of each sex were subjected successively to four 14-day test periods with densities of 4, 8, 24 and then back to 4 animals per pen; (*c*) mixed-sex groups with one male : three females per pen were compared with groups containing two males : two females. High-density animals in test series *a* selected about 70 per cent (males) and 90 per cent (females) more of the NaCl solution than the low-density animals (*P* less than 0·01, paired *t*-test). In test series *b*, NaCl selection varied directly with crowding when density was changed on the same twenty-four animals. This was most striking in males, where the ratio of NaCl solution to distilled water selected increased from about 2:1 successively to 6:1, 10:1 and back to about 4:1 with the return to low density. In test series *c*, groups consisting of two males and two females selected a significantly higher ratio of NaCl solution to distilled water (6·2:1) than did the groups of one male and three females (2·1:1).

Increased appetites for sodium shown by adrenalectomized laboratory animals have been considered to reflect an increased physiological need for this salt[1–3]. This suggests that the higher selection of NaCl by our animals under conditions of continued crowding was a manifestation of sodium deficiency, perhaps due to inadequate adrenocortical regulation of sodium metabolism. The behavioural and physiological mechanisms by which a sodium deficiency might affect reproduction in crowded animals is beyond the scope of this report.

The field data and laboratory tests described above suggest that with restricted access to salt the growth of vole populations may be checked at relatively low levels by physiological responses associated with crowding, and that with unrestricted access to salt the suppressive affects of crowding are alleviated, allowing populations to increase

to higher levels. Implied in these deductions is the principle that sodium is the critical factor limiting vole populations in many areas. In regions with high sodium soils, enough salt may be ingested with the vegetation to satisfy the increased requirements of crowded animals, thereby permitting unrestricted reproduction and population growth to the point where food or some other factor becomes critical.

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Simple Technique for embedding and supporting Delicate Biological Specimens

Many biological specimens are too large for treatment by normal microscopical techniques and, at the same time, too small and delicate to withstand very much manipulation. Many embedding media have been tried over the past few years, and one of the most efficient and effective is agar.

A 4 per cent aqueous (distilled water) solution of agar ('Bacto', Difco Laboratories) is prepared, either by free steaming in an autoclave or steamer, or by using a beaker suspended in a boiling water bath. The molten agar is cooled to about 40° C and a thin layer poured into a solid watch-glass and allowed to set. The specimen is arranged on the surface of the agar and is then covered by a further layer of molten agar. It is important to ensure that no moisture is left on the first layer. Moisture globules will spread between the two layers and can cause separation after setting. This trouble is obviated by wiping the specimen and the surface of the agar with a small piece of filter paper after the specimen has been orientated. When the agar has set, the lens-shaped block is eased out of the watch-glass and the part containing the specimen trimmed into a cubical block, using a sharp scalpel or razor blade. The cube (which is opaque when set) is then transferred to 70 per cent alcohol, in which it may be stored until required for clearing. For clearing, the block is gradually dehydrated by being taken through 80 per cent, 90 per cent and absolute alcohol, one day in each, and then transferred to a mixture of equal parts of absolute alcohol and benzyl alcohol for a few hours. It is then removed to pure benzyl alcohol for complete clearing. Both block and specimen will become transparent quite quickly but specimens containing a great deal of yolk may require a longer time to achieve complete transparency. In the case of amphibian eggs this is an advantage because the slight opacity gives a clearer picture of the segmentation stages.

The technique is particularly useful for eggs of cyclostomes, teleost fishes and amphibia, coelenterate medusae, dissections of small molluscs and molluscan radulae too large for ordinary microscope mounts. Specimens may be stained before embedding, but they must be returned to water before processing. Blocks may be removed from the benzyl alcohol for examination under a dissecting microscope, but should not be kept in the air for a period of more than 3 h, otherwise splitting and drying of the block may occur. All material for treatment under these conditions must first be fixed and preserved as for normal storage. The blocks are tough and resistant to wear, but should it be necessary to remove a specimen from a block a careful cut across a block in one plane will enable the specimen to be removed without damage. All parings from the first trimming may be remelted and used for more blocks. Agar cannot be recovered after treatment with alcohol.

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Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna

Simulium, the vector of onchocerciasis and the cause of considerable nuisance in northern latitudes, is usually controlled by killing its aquatic stages by putting insecticides in a soluble form into the streams where they occur. This kills most of the other insects; the natural predatory suppressors of the *Simulium* larvae—the larvae of some of the stone flies and caddis flies—are removed, and unrestrained recolonization by *Simulium* may occur.

The community of creatures living in fresh-water streams is highly organized and stable and constitutes many food chains, one ending in fish. The use of soluble DDT necessitates repetition and produces an entirely different organization, the maintenance of which is dependent on the maintenance of the interference. This changed environment can be regarded in many respects as biologically sterile.

Simulium larvae are particle feeders. They live only in the fast-flowing parts of the stream, and their method of feeding is different from that of the other detritus feeders such as the chironomids, which live in the slow-running and silted part of the stream. The size of particles ingested by British species of *Simulium* is about 10–12μ (ref. 1). Since 1962, we have used DDT in the form of particles of different size in streams in North Wales. In one such experiment, 1×10^{12} particles of 4–15μ were put into a stream in half an hour, when the stream was flowing at a rate of 0·15 cu. ft./sec. This corresponded to a dosage of 0·5 p.p.m. The next day, all *Simulium* larvae had disappeared from polythene tapes placed 150 yards below the point of dosage, and the tapes remained free of *Simulium* until a month after the dosing. During this time no other creatures in either the water or the stream bed showed any population variation other than the minor fluctuations expected from the changing seasons and rainfall. Not even net-spinning caddis fly larvae were affected by the small particles of DDT.

It is possible that a method of control developed on this principle will not disturb the predator insects which regulate the numbers of *Simulium* larvae, and it should not interfere with the tightly knit community of creatures which includes the food chain leading to fish. But we do not know whether the particulate insecticide accumulates in the sediment of the river bed, nor do we know anything of the potential effects of such accumulation, and we know nothing of the fate of that fraction which is taken up by *Simulium* larvae. These and other factors must be investigated.

We wish to thank "Shell" Research, Ltd., Woodstock Agricultural Research Centre, for providing water dispersible powders of the required particle size.

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MICROBIOLOGY

Bacterial Count and Cell Differentiation in Milk

A method has been developed in this laboratory which has been found to be superior to the Breed-type smears such as are routinely used for assessing the bacteriological quality of cow's milk. The technique not only stains the bacteria, but also the somatic cells in such a way that the various cell-types may be satisfactorily differentiated and classified. Granular cytoplasmic inclusions which might be released on cell-degeneration are easily distinguished and not mistaken for the coccoid form of micro-organisms.

The use of 0·2 ml. of sample instead of 0·01 ml. (as in the Breed-method and its modifications), even distribution and low microscopic factor improves the precision of the count.

The fact that the cells are differentiated will help the investigator who is not only concerned with a total count to distinguish between pathological and normal conditions of the udder. The micro-organisms and the cells present in the milk sample are stained in a siliconed serological tube. This stained preparation is filtered, by vacuum filtration, through a membrane filter (M.F. Corp. plain white D.A.; diam., 25 mm; pore size, 0·65μ). This filter, after being dried, is made transparent with immersion oil and the bacteria and cells can then be counted and studied with a light microscope.

The procedure is as follows: 2 ml. 'Triton *X*-100', 0·1 per cent solution, *p*H 7·0 (prepared with double distilled water and filtered through a membrane filter), is placed in a serological tube. 2–3 drops 0·5 per cent solution $CuSO_4 5H_2O$ is added. Three drops benzidine–peroxide solution is added (benzidine 0·2 g/200 ml. distilled water; to which is added 4 drops of H_2O_2, 3 per cent). The milk sample (0·2 ml.) is added, and the mixture allowed to stand for 3 min. One drop of May–Grünwald stain (BDH standard stain in solution) is added, with 2 drops of Giemsa stain (improved Giemsa stain, Gurr *R*66 in solution). The tube is stood for 20 min in a water bath at 48° C, then poured into a filter funnel; two rinsings of the serological tube with 'Triton' solution at 48° C are also poured into the funnel. Vacuum is applied to pull the emulsion through the membrane filter. Rinsing is accomplished by flushing about 7 ml. 'Triton' solution (at 48° C) through the filter. The membrane filter is removed and dried on a glass slide at 35° C for about 10 min; immersion oil is added until the membrane filter becomes transparent. It is then covered with a cover slip. Another drop of immersion oil is placed on the cover slip, and the preparation is examined under the microscope.

I thank Prof. A. G. Leggatt for his advice.

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Formation of Protocatechuic from Quinic Acid by Fluorescent Pseudomonads

Rogoff[1] described two fluorescent pseudomonads he electively isolated from soil which could oxidize quinic acid with the formation of protocatechuic acid as an intermediate.

Fifty-two isolates, assignable to the genus *Pseudomonas* and producing pyoverdine in the medium of King, Ward and Raney[2], have been examined for their ability to aromatize quinic acid by this pathway. The strains were isolated from infected hen-eggs, surface waters, chicken carcases, chicken droppings, beef and milk, using ordinary peptone media containing no quinate.

Protocatechuate formation by the isolates was investigated in the following medium, adapted from that of Rogoff[1]: *Solution A*. NH_4Cl, 0·4 g; $MgSO_4.7H_2O$, 0·05 g; $MnCl_2.4H_2O$, 0·01 g; $FeSO_4.7H_2O$, 0·005 g; $CaCO_3$, 0·2 g; yeast extract (Difco), 0·05 g; agar No. 3 (Oxoid), 0·05 g; distilled water, 50 ml., *p*H 7·4. *Solution B*. K_2HPO_4, 0·1 g; quinic acid, 1·0 g; distilled water, 50 ml., *p*H 7·4 (adjusted with 10 per cent (w/v) NaOH). The two solutions were mixed, dispensed in 2-ml. amounts into test-tubes (100 mm × 10 mm) and autoclaved for 15 min at 15 lb. pressure. One drop from broth cultures of the 52 strains was used to inoculate the quinate medium. On incubation at 25°, protocatechuate was readily detected by the formation of a purple colour below the bacterial surface growth due to the action of ferrous ions on this compound. In most such cases, a green coloration also appeared at the medium surface: this may have been due to the reaction of protocatechuate with ferric ions, formed at the medium surface by oxidation of ferrous ions (Soloway and Rosen[3]), but since this zone fluoresced strongly it may simply have been pyoverdine produced on the surface of the medium. A drop of medium removed from the purple portion of each culture did, however, form a deep green colour on the addition of a drop of aqueous 0·5 per cent (w/v) $FeCl_3.6H_2O$. By these simple tests, protocatechuate was discernible in the cultures within 2–3 days; after a week or so, the purple colour slowly disappeared and no ferric chloride reaction could be demonstrated.

Of the 52 fluorescent pseudomonads, 49 produced protocatechuate. The three negative strains (from river water, chicken droppings and spoiling beef respectively) grew more sparsely on the medium, utilizing the yeast extract as a carbon source, presumably. An interesting biochemical characteristic these three strains shared was the ability to decarboxylate L-glutamic acid to γ-amino butyric acid; one of the other strains was also able to carry out this reaction but it possessed in addition an L-aspartic acid decarboxylase.

Manometric experiments with washed cell suspensions gathered after 60 h growth on the quinate medium (solidified with 1·5 per cent w/v agar) demonstrated no oxygen uptake by the three strains in the presence of sodium quinate (*p*H 7·4, 30°), whereas representatives of the other 49 strains rapidly oxidized this substrate.

It would seem that fluorescent pseudomonads commonly possess the ability to oxidize quinic acid via protocatechuic acid but that some strains are unable to attack it by this or any other pathway. Since the formation of protocatechuate can be easily demonstrated in growing cultures by its reactions with ferrous and ferric ions, and since a significant number of strains do not effect its production, the test should prove a useful one for inclusion in taxometric methods for the sub-grouping of the *Pseudomonas*. It has been found that the use of a semi-solid medium favours the accumulation of the protocatechuate formed in the region below the surface growth and prevents its too rapid oxidation.

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VIROLOGY

Rubella Virus Complement-fixation Test

Methods in present use for the serological diagnosis of rubella consist of the neutralization[1,2] and the indirect fluorescent-antibody[3] techniques. The former is expensive and laborious and requires usually 7–10 days to carry out, while the latter, although it can give an answer within a matter of hours, is a difficult technique for routine

diagnosis. The preparation of a complement-fixing antigen has recently been described[4], providing for a rapid and simple test of infection. The antigen, which is cell-associated, was made in the RK_{13} line of rabbit kidney cells or in primary cultures of kidney from the African green monkey. The infected cells were processed 2 days and 7–10 days respectively after inoculation of virus. Successful antigen preparation, however, is in both cases dependent on a critically large virus inoculum; otherwise results are erratic. The present report describes a simpler and more reliable method of complement-fixing antigen preparation, involving the use of aged cultures of a chronically infected line of $LLC\text{–}MK_2$ cells[5].

The chronically infected cells were grown in Roux bottles in Medium 199 containing 1 per cent inactivated horse serum and antibiotics and adjusted to *p*H 7·4 with sodium bicarbonate. The cultures were maintained by replacing half the medium every 3–4 days. Antigen was prepared at intervals by discarding the medium and scraping the cells from the glass into a small volume of veronal-buffered saline. The cells were sedimented by centrifuging at 1,000 r.p.m. for 10 min, and resuspended in buffer to a final volume equivalent to 1 ml. per Roux bottle. This cell suspension was then frozen and thawed three times, or subjected to ultrasonic vibrations for 15 sec. The final product constitutes the antigen. The technique is similar to that used in preparing cytomegalovirus complement-fixing antigen[6]. The antigen can be stored at −70° C for at least 1 month without loss of potency. Activity is lost at 56° C within 0·5 h. Because of the relatively small amounts of antigen available, complement-fixation tests were carried out by the micro-technique, using 0·025-ml. Takatsy loops, micropipettes and 'Perspex' plates[7]. Each batch of antigen was examined in a chess-board titration against a positive control convalescent serum, using 2 units of complement and overnight fixation at 4° C.

Little or no antigenic activity was demonstrable in preparations from cell cultures less than 7 days old. Antigenicity first became detectable with 7–14-day-old cultures but the titres, measured as the reciprocal of the greatest dilution of antigen showing at least 3 + complement fixation with the positive serum, did not exceed 2. Maximum antigen titres of 8 were, however, reached after 24 days of incubation of the cell cultures. The material showed no or only minimal anticomplementary activity when used undiluted. While Sever *et al.*[4] briefly mention in their paper the preparation of complement-fixing antigen from the chronically infected $LLC\text{–}MK_2$ cells, they apparently found no advantage with this technique and no details are given. The present findings indicate that with aged cells as described here the antigen potency is at least equivalent to that produced in RK_{13} and primary African green monkey kidney cells. Although prolonged incubation of the chronically infected cells is required for antigen manufacture, the initial preparation of large inoculum doses of virus becomes unnecessary and, in our experience, potent antigens are obtained regularly.

Table 1 shows the results observed with a number of sera from children and adults. All sera for complement-fixation tests were inactivated by 56° C, and were titrated against 2 units of antigen. The tests were controlled with known positive and negative sera. Neutralizing antibody titres were measured in RK_{13} cells. Serial dilutions of non-inactivated sera were mixed with equal volumes of a suspension of the Judith strain of rubella virus containing 100 TCD_{50} per 0·1 ml., and the mixtures were incubated at 37° C for 1 h. Tissue culture tubes were then inoculated with 0·2 ml. amounts of the serum–virus mixtures, and incubated at 33° C in a roller drum. The tubes were examined for cytopathic effects over a period of 14 days. The neutralizing antibody titres were calculated as the reciprocal of the highest dilution of serum which completely suppressed the cytopathic effect of the virus.

Table 1. Antibody Responses in Patients with Rubella Virus Infection

Age	Time of serum collection	Antibody titres CF	Neutr.
Patients with clinical rubella			
18 yr.	First day*	< 4	40
	21 days later	64	320
20 yr.	Second day*	< 4	20
	24 days later	32	160
3 yr.	Third day*	< 4	20
	7 days later	32	80
	23 days later	64	160
6 yr.	Third day*	< 4	20
	20 days later	64	160
18 yr.	Sixth day*	16	80
	17 days later	64	80
Infants with rubella syndrome			
4 wk.†		< 4	20
Mother		128	640
8 mos.‡		32	80
Mother		128	320
2·5 yr.§		< 4	80
Adults with no or past history of rubella			
25 yr.		< 4	160
24 yr.		< 4	40
23 yr.		< 4	20
21 yr.		< 4	80
20 yr.		4	40

* After onset of rash.
† Virus isolated from throat and faeces.
‡ Virus isolated from urine and throat.
§ Not excreting virus.

While only a few cases of clinical rubella have so far been investigated with the complement-fixation test, it is apparent that the complement-fixing antibody develops in parallel with the neutralizing antibody but is delayed by several days. The latter antibody is already detectable in most cases by the second day after the onset of the rash[1,8,9], whereas the complement-fixing antibody probably does not appear until about the fifth day. The presence of neutralizing antibody without complement-fixing antibody in adults who give no recent history of rubella suggests that the complement-fixing antibody is transient[4]. The neutralization test will therefore still be necessary for the detection of past infection and for epidemiological studies. The three cases of rubella syndrome illustrate three different and interesting antibody situations. The first case possesses only neutralizing antibody, despite active infection and virus excretion. However, inability to make complement-fixing antibody is not uncommon in small infants in many other virus infections. The 8-month-old case shows both types of antibody, perhaps because of greater maturity and continued infection. The 2·5-year-old case has ceased to excrete virus, and no longer possesses demonstrable complement-fixing antibody. This disease differs, therefore, from cytomegalovirus and herpes virus infections, and the findings in this child appear to suggest that there is no persistent latent infection with rubella virus in children with the rubella syndrome.

The complement-fixation test provides an easy and rapid method of serological investigation of recent rubella virus infection, and should contribute to a more complete understanding of the pathogenesis and epidemiology of this common and important disease.

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GENETICS

Phenocopies of the *ma-l* and *ry* Mutants of *Drosophila melanogaster*: Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo(3,4-d)pyrimidine

Phenocopies are environmentally induced abnormalities which mimic the phenotype resulting from mutant genes. Pomales *et al.*[1] have shown that 4-hydroxypyrazolo (3,4-d)pyrimidine (HPP) is an inhibitor *in vivo* of xanthine oxidase in mice, and we have attempted to produce phenocopies of the maroon-like (*ma-l*) and rosy (*ry*) eye colour mutants of *Drosophila melanogaster* with it. Xanthine dehydrogenase is undetectable in these mutants (see Glassman[2] for a discussion of these loci), and as a result, there is a partial loss of the red pteridines of the eye, the eyes being a dark red-brown rather than red. In addition, the products of this enzyme's activity, isoxanthopterin and uric acid, cannot be detected, and its substrates, 2-amino-4-hydroxypteridine and hypoxanthine, accumulate. In this communication we describe the production of phenocopies of *ma-l* and *ry* by HPP in various strains of *D. melanogaster*.

The medium on which the flies were raised was modified from one described by Dr. E. B. Lewis (personal communication). 100 ml. medium was prepared by adding 1·7 g brewer's yeast, 5·7 g dextrose, 1·4 g sucrose, 9·4 g fine-ground yellow corn meal, 0·6 g agar, and 1·0 ml. food colouring (for identification) to 94 ml. water at 85° C. The mixture was heated to 95° C until it started to thicken. When it had cooled to 70° C, 1·2 ml. of 3 per cent benzyl benzoate (Eastman) in 95 per cent ethanol (to control mites), 1·0 ml. of a mixture containing 41·8 ml. propionic acid, 4·15 ml. phosphoric acid (85 per cent), and water to 100 ml. (to control mould), and the desired amount of HPP were added. The medium was then mixed in a Waring blender and 10-ml. portions were dispensed into 25 × 95 mm vials. The next day, adult flies were placed in each vial and allowed to deposit eggs on the surface of the medium for 3 to 4 days. Flies were reared at 25° ± 1° C.

Eye colours were recorded daily when the adults emerged. In order to facilitate this a strain containing the scarlet eye colour gene (*st*) at locus 44 on the third chromosome was used. The *st* mutant lacks the brown eye pigment and the eye colour is usually bright red. The double mutant, *st ry* or *ma-l*; *st*, has a yellow-orange eye colour, and this is also the eye colour of the *st* flies which are phenocopies of *ma-l* and *ry*. For comparative purposes, the Pacific wild-type strain and the *ru lxd by* strain was also used (*ru* and *by* are the symbols for roughoid eye and blistery wing, and are only incidental markers). The *ru lxd by* strain has relatively low xanthine dehydrogenase activity because of *lxd* (low xanthine dehydrogenase) at locus 33 ± on the third chromosome[3]. Only those flies that duplicate the *ma-l* or *ry* eye colour exactly (red-brown eye colour in the case of Pacific and *lxd*, and orange eye colour for *st*) were scored as phenocopies; those showing intermediate eye colours were scored as normal.

Fig. 1 shows the percentage of flies grown on different amounts of HPP that resembled the *ry* and *ma-l* mutants. In no instance did any of these phenocopy flies transmit their abnormal eye colour to their progeny grown on regular food. The concentration of HPP required to transform 50 per cent of the flies into phenocopies varied. For *ru lxd by* it was about 0·008 per cent, for *st* it was about 0·026 per cent, and for *PAC* it was about 0·095 per cent. This difference in response might be due to the average xanthine dehydrogenase activity found in these strains; for the *ru lxd by* it was 3·8, for *st* it was 10·0 and for *PAC* it was 22·4 enzyme units per fly as measured by the method of Glassman[3,4]. However, additional work is required to substantiate this relationship. Higher doses of HPP have an appreciable detrimental effect on viability (Table 1).

Table 1. The Effects of HPP on the *st* Strain of *Drosophila melanogaster*

Conc. of HPP	% Flies with phenocopy eye colour	Relative survival	Relative amount of isoxanthopterin*	Xanthine dehydrogenase units per fly†
0·0	0	1·00	4·0	12
0·01	2·4	0·84	3·2	9·6
0·02	3·1	—	—	10
0·03	93·8	—	—	9·4
0·04	100·0	—	—	8·6
0·05	100·0	0·81	2·2	11
0·10	—	0·02	0·5	—

* Data for *st*, *PAC*, and *ru lxd by* flies are combined for this column.
† The mean of 8 replicate assays of single flies.

To test whether the other pteridines (2-amino-4-hydroxypteridine and isoxanthopterin) related to xanthine dehydrogenase were also affected, flies were chromatographed by the following modification of the method of Hadorn and Mitchell[5]. A fly was placed in boiling water for 1 min and then squashed with a glass stirring rod on the origin of a 9 in. × 11 in. sheet of Whatman No. 3 *MM* filter paper. The chromatograms were developed by ascending chromatography for about 4 h in propanol : 1 per cent NH_3OH (2 : 1). When dry, the chromatograms were observed under an ultra-violet lamp emitting mainly at 365 mμ. In order to quantitate these results, we applied an arbitrary visual grading system using '0' to represent the absence of isoxanthopterin and '4' to represent the highest amounts we observed. The grading was done on coded flies to eliminate bias. The data are presented in Table 1, where it can be seen that the amount of isoxanthopterin declined as the concentration of HPP increased. In contrast, 2-amino-4-hydroxypteridine was present in higher-than-usual amounts in the flies raised on the higher concentration of HPP. This indicates that the effect of HPP is on xanthine dehydrogenase and not directly on the eye colour or on pteridines.

Furthermore, HPP has no effect on the synthesis of this enzyme. Table 1 shows that the enzyme activity was not affected in *st* flies grown on HPP even though the flies grown on 0·030, 0·040 and 0·050 per cent HPP were phenocopies. It is concluded that the synthesis of xanthine dehydrogenase is not affected by HPP.

It is clear that HPP effectively inhibits xanthine dehydrogenase activity in *D. melanogaster*, *in vivo*. Furthermore, the effectiveness of HPP seems to be related inversely to the amount of enzyme activity present in each strain. This phenomenon will be useful in many ways: (1) by suppressing the maternal effect of the *ma-l*$^+$

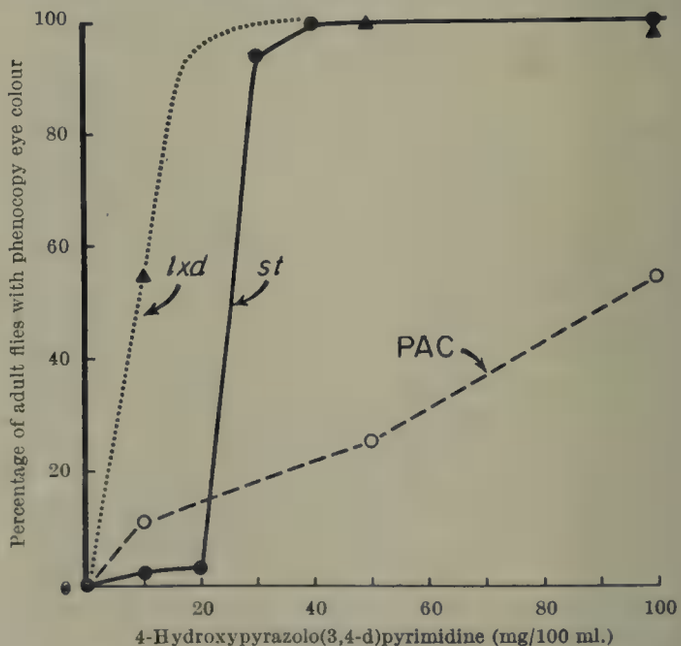

Fig. 1. The effect of HPP on phenocopy production in the *lxd*, *st*, and *PAC* strains of *Drosophila melanogaster*

gene[6,7] with a low concentration of HPP in the food, we can expedite the genetic analysis of the *ma-l* locus; (2) by using low concentrations of HPP in food and using the usual genetic selection techniques, it might be possible to select for new mutant alleles at the *lxd* locus, or for leaky mutants at the *ma-l* and *ry* loci; (3) the use of high levels of HPP in the food may facilitate the selection of strains of flies that have very high xanthine dehydrogenase activity. These applications are now being explored.

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Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract

The frequency of gene recombination is known to be affected by extrinsic factors or intrinsic ones such as sex. Crossing-over is completely blocked in dipteran males, including those of *Drosophila melanogaster*, and in silkworm females. It is a puzzling problem why sex should have such an influence on crossing-over. Lack of homology between regions of the *X* and *Y* may explain the absence of crossing-over between the sex-chromosomes of the heterogametic sex, but the absence of crossing-over in the autosomes must have a different cause.

All agents that so far have been found to produce crossing-over in *Drosophila* males—irradiation and certain chemicals—cause mutations and chromosomal aberrations as well. In the work recorded here, an attempt has been made to induce crossing-over in males by means of fresh ovarian extracts. Males and females utilized in this work were heterozygous for the second-chromosome markers dumpy (*dp*), black (*b*), cinnabar (*cn*), and brown (*bw*). About 300 ovaries of fertilized females were dissected into 0·3 c.c. of 0·4 per cent saline. The ovaries were then homogenized and centrifuged twice at 3,000 r.p.m. for 15–20 min. The supernatant was filtered and/or directly taken into a microsyringe, and 0·2 μl. was injected into the testis region of males. A portion of the same or a similarly obtained supernatant was boiled before injection. Males injected with 0·4 per cent saline and untreated males served as additional controls. 24 h after injection, each male was individually mated with three virgin females homozygous for *dp b cn bw*, and six broods of three days each were analysed for induced cross-overs in F_1. Presumptive cross-overs were verified by back-crosses to the multiple recessive strain.

Table 1 presents a summary of the results. It will be seen that cross-overs occurred exclusively among the progeny of a proportion of those males that had been injected with fresh ovarian extract.

Table 2 presents an analysis of the progeny from the 25 males that had yielded cross-overs. The overall frequency of crossing-over was 5 per cent. The distribution over the 3 chromosomal regions used did not correspond to the lengths of these regions, and there were great disparities between complementary classes; these unexpected features may, however, be due to the fact that sampling errors were greatly exaggerated by the occurrence of clusters. The brood pattern shows that, as in the experiments by Reddi and Auerbach[1], some of the spermatozoa utilized in brood *B* must have been pre-meiotic or in early meiosis at the time of treatment. The occurrence of some clusters in this brood would suggest the former possibility but, with the few markers used, apparent clusters may equally well have been due to independent cross-overs in several spermatocytes of the same male.

Table 1

Injected with	No. surviving males	No. F_1 tested	No. cross-overs in F_1
—	87	14,472	0
Saline	95	14,043	0
Boiled ovarian extract	69	11,530	0
Fresh ovarian extract	194	29,147	103*

* Among 1,901 progeny of 25 males.

Table 2. Distribution of Cross-overs in the Progeny of 25 Males that had been treated with Ovarian Extract

Brood	Observed phenotypes	*B*	*C*	*D*	*E*	Total
Non cross-overs	+ + + + / *dp b cn bw*	897	675	196	108	1,876
Cross-over regions:						
I (*dp* – *b*)	+ *b cn bw*	9	3	6	12	30
II (*b* – *cn*)	+ + *cn bw*	1	3	–	–	4
III (*cn* – *bw*)	+ + + *bw*	13	5	2	–	20
	dp b cn +	2	3	–	1	6
I and II	*dp* + *cn bw*	1	–	–	–	1
	+ *b cn* +	1	–	–	–	1
I and III	*dp* + + *bw*	7	1	–	–	8
II and III	+ + *cn* +	16	12	–	–	28
	dp b + *bw*	–	1	–	–	1
	Numbers	50	28	8	13	99
Total cross-overs	per cent	5·5	4	4	11	5

While the details of the observed effect require a more elaborate analysis, the results clearly indicate that fresh ovarian extract contains a factor that induces crossing-over in male germ cells. The ineffectiveness of boiled ovarian extract suggests that this factor is a heat-labile substance.

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Regression of Phenotypic Value on the Values for the Parents and Grandparents

The problem discussed in this communication was suggested by Dr. Donald Michie in connexion with an investigation, by himself and Mrs. Jean Hayes, of pedigree records in British bloodstock.

Let x be some phenotypic value for some animal, and let x^P be the average of the value for its two parents, and x^G that of its four grandparents. The problem is to find the regression of x on x^P and x^G, that is to find the partial regression coefficients $b_{HG.P}$ and $b_{HP.G}$ such that:

$$E(x|x^P, x^G) = b_{HP.G}x^P + b_{HG.P}x^G.$$

Write :

$$x = w + z,\ x^P = w^P + z^P,\ x^G = w^G + z^G,$$

where the w's are 'environmental values' and the z's are 'genotypic values'. We make the following assumptions, the first of which implies no real loss of generality, and the remainder are natural.

$$E(x) = 0,\ E(w) = E(z) = 0,\ \mathrm{cov}(w,w^P) = \mathrm{cov}(w,w^G) = \mathrm{cov}(w,z) = \mathrm{cov}(w,z^P) = \mathrm{cov}(w,z^G) = 0.$$

We also assume that the conditions are stationary from generation to generation, and that:

$$E(z|z^P) = E(z|z^P, z^G) = z^P.$$

This last assumption means that the breeding (genotypic) properties of the parents supersede those of the grandparents, at any rate in regard to expectations.

Let h be the correlation coefficient between x and z. It is customary to call h^2 the 'heritability' of the relevant trait. It can then be proved, by making use of standard regression theory, that:

$$b_{HP.G} = h^2(1-\tfrac{1}{2}h^2)/(1-\tfrac{1}{2}h^4)$$

$$b_{HG.P} = h^2(1-h^2)/(1-\tfrac{1}{2}h^4)$$

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AGRICULTURE

Objective Evaluation of Quality in Poultry Meat

Recent work in this laboratory[1-5] has indicated a relation between biochemical and quality changes occurring in chicken muscle during storage. During frozen storage the buffer-extractable nitrogen of chicken muscle decreased and the products of protein breakdown increased[1]. The decrease in buffer-extractable nitrogen occurred as a result of loss of solubility of the actomyosin fraction and was accompanied by a loss of sulphydryl groups (–SH) of muscle tissue. During storage at above-freezing temperatures, extensive proteolysis occurred, but the changes in the protein extractability and –SH group content of muscle tissue were small[2]. In meat in which the proteolytic activity had been destroyed by cooking, the –SH groups of muscle tissue decreased progressively during frozen storage without any increase in the protein-breakdown products[3]. The loss of –SH groups and solubility of muscle protein was accompanied by a loss of tenderness and development of dryness in frozen stored meat, while the amount of protein-breakdown products increased with the development of off-odour in meat stored either frozen or unfrozen.

Since both biochemical and quality changes depend on storage time and temperature, the possibility of employing the biochemical changes as an index of quality in stored poultry was considered. This communication presents evidence that the ratio between the –SH groups of muscle tissue, which indicate the degree of denaturation of muscle proteins, and protein-breakdown products, which indicate the degree of proteolysis, may be useful as a quality index for poultry stored frozen or unfrozen and for cooked frozen poultry. Separation and fractionation of myofibrillar proteins are lengthy procedures and cannot be used for routine quality control work, but simple analytical methods can be used to measure these parameters.

Samples of meat were obtained from male broiler-type chickens. The birds were killed in the laboratory by cutting the jugular vein and carotid arteries. They were bled for 2–3 min, scalded for 2 min at 53°–54° C, plucked by hand, eviscerated and aged for 48 h in drained, crushed ice. For frozen storage, birds were vacuum packaged in 'Cry-O-Vac' bags, frozen in an air blast (300–500 ft./min) at − 30° C, stored at − 18 and − 10° C for the desired length of time (up to 2 years), and thawed for 2 h in running water at 18°–22° C. For storage at 0° C, birds were dipped in chlortetracycline solution (10 p.p.m.), placed in sterilized 'Cry-O-Vac' bags, flushed with nitrogen and stored under aseptic conditions for maximum storage life (up to 5 weeks). For pre-cooked frozen storage, the birds were vacuum packaged in 'Visking' bags, heated for about 3 h in boiling water to bring the internal temperature to about 85° C and stored at −10 ° C (up to 2 years) under nitrogen to minimize oxidation.

For analysis, breast and leg muscle were removed separately, freed from visible fat, tendon and connective tissues and minced with scissors. Minced tissue (5 g) was homogenized with sodium chloride (3 g), sand (3 g) and metaphosphoric acid (2·25 per cent, 20 ml.). The mixture was shaken, left for 5 min and filtered (Whatman No. 40). These operations were performed at 0° C on duplicate samples. The filtrate was used to estimate both –SH groups and protein-breakdown products. Sulphydryl groups were estimated in 2–4 ml. of filtrate by a colorimetric method using sodium nitroprusside reagent[1] and expressed as μg glutathione–SH per g muscle. For the estimation of protein-breakdown products, 5 ml. filtrate was treated with 5 ml. 20 per cent trichloroacetic acid solution, allowed to stand at room temperature for 1 h and centrifuged for 10 min at 12,000 *g*. Protein breakdown products were estimated with Folin-Ciocalteu reagent in 4 ml. supernatant[1,2] and expressed as μg of tyrosine nitrogen per g muscle.

The ratio of –SH groups and tyrosine nitrogen content (suggested quality index) of both breast and leg muscle tissue decreased progressively with storage time in raw meat stored at temperatures above and below freezing, and in cooked frozen meat (Fig. 1). During frozen storage the 'quality index' decreased as a result of loss of –SH groups and an increased proteolysis. During storage at 0° C for 5 weeks, changes in –SH group content of muscle tissue were small, and the 'quality index' decreased mainly as a result of proteolysis. In cooked meat in which no proteolysis occurred, the 'quality index' decreased as a result of loss of –SH group content during frozen storage. The rate of decrease of 'quality index' increased rapidly with storage temperature in much the same way as the rate of quality deterioration increased in studies with uncooked meat[5,6]. In muscle of birds from a single flock killed, processed, and stored under identical conditions in the laboratory, the 'quality index' varied within ±10 per cent.

The determination of the suggested objective 'quality index' should be much simpler, more accurate and reliable than the assessment of quality by sensory testing. The results may, therefore, be of interest in laboratory studies on the effect of freezing and storage procedures on poultry quality as well as in commercial freezing and storage of poultry. The effects of biological variability and processing techniques on the 'quality index' are being investi-

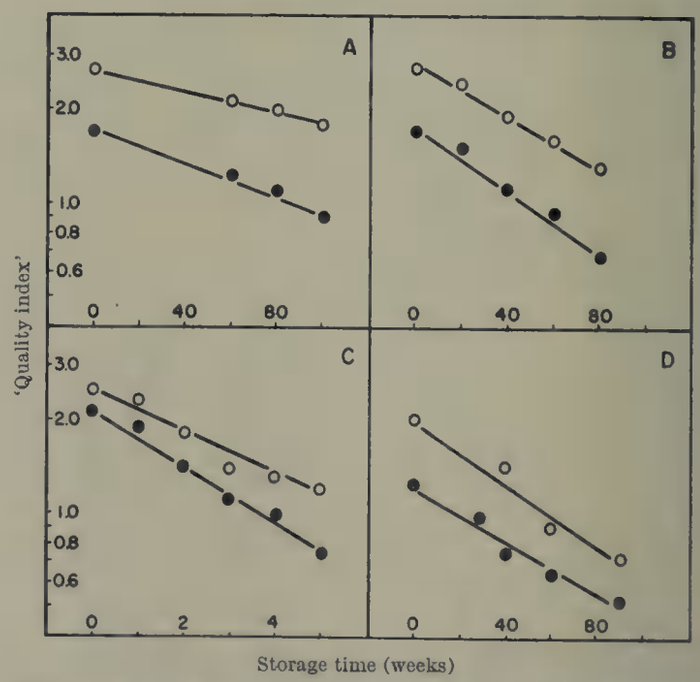

Fig. 1. Effect of storage time on suggested quality index (ratio between –SH group content and Folin-Ciocalteu reagent-positive nitrogen content of muscle tissue, plotted logarithmically). ●, Breast muscle; ○, leg muscle. *A*, Raw poultry stored at −18° C; *B*, raw poultry stored at −10° C; *C*, raw poultry stored at 0° C; and *D*, cooked poultry stored at −10° C

gated, and values of the 'quality index' corresponding to the quality of meat when deterioration is first detected by sensory evaluation are being studied.

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ANTHROPOLOGY

Lower Palaeolithic Hominid and Pebble-industry in Hungary

In the neighbourhood of the village of Vértesszöllős, to the north-west of Budapest, a rich Lower Palaeolithic site has been found: the results of the first excavation have already been published[1].

In 1964 a great part of the site was excavated, and this resulted in some new finds, which included a rich fauna as well as several thousand primitive pebble-tools.

The pebble-industry in Hungary, called *Buda*-industry, is related to the Oldowan industry in Africa, the Choukoutienian in China, the Sohan in India and to the Clactonian in England so far as implement-types is concerned. The most characteristic forms are pebble-choppers, chopping-tools and polyhedrons. On the basis of its well-determinable stratigraphical conditions (the travertine complex bearing the finds is situated on a high, old—possibly fourth—Pleistocene terrace) and of the rich vertebrate fauna, it was possible to compare our site with the Upper Biharian (that is, Mindel 1/2 and Mindel 2).

During the 1964 excavations four separate cultural horizons were found. The two lower ones were in solid travertine and in lime-mud, while the upper two were at the base and at the top of the overlying loess. The loess again was covered with another layer of travertine several metres thick. It was the lowermost layer which yielded the richest material; this represented a continuous living surface at the base of a former tettarata (calcareous tuff basin). The material consists of numerous broken bones, and some small hearth, stones and chips. Of the three upper layers, that at the base of the loess was almost as rich in material as the lowest layer, but it covered a smaller area. The tools—so far as can be stated after a preliminary investigation—do not show any noticeable changes at the different cultural horizons. However, the fauna shows a considerable change: the vertebrate (rodent) material found in the loess belongs to cold-enduring species while those in the lower layers belong to a warmer climate: in the upper layer, beside the northern type red vole (*Myodes* [=*Clethrionomys*] cf. *rutilus*), there are *Microtus*- species and *Ochotona*, etc., in great abundance, thus indicating a cold climate. On the other hand, in the lower layers, Murines (*Apodemus*, *Mus*), *Cricetus* and *Glis* accompanied by *Pliomys* (dying out later), and Microtines, which belong to a relatively warmer climate, indicate more temperate climatic conditions.

During the excavation, several hundredweights of lime-mud from the cultural layer were washed out and sieved, and taken to the Hungarian National Museum for preparation. The washing process and the collecting from the washed material took several months. It is for this reason that the most significant find of the excavation only came to light early in 1965: namely, tooth-remains of a hominid from the washed material of the lowest cultural horizon. The hominid remains consist of a left deciduous canine, the fragment of a first deciduous molar and fragments of another molar belonging to a child's jaw. Even at first glance it was obvious that the teeth are characteristic of the *Pithecanthropus–Sinanthropus* group. Dr. A. Thoma has been asked to undertake detailed examination.

From the washed material where the human teeth were contained, the following mammalian species were also determined (there are only one or two new species in this list as compared with that already published): *Citellus* sp. (*citellus*-group), *Cricetus cricetus* ssp., *Myodes* [=*Clethrionomys*] cf. *glareolus* (*Schreber*), *Pliomys* sp., *Arvicola* sp., *Phaiomys* sp., *Microtus* (*Pitymys*) *arvalidens* (Kretzoi), *Microtus conjungens* Kretzoi, *Microtus arvalis-agrestis*-group, *Apodemus* sp. (*sylvaticus*-group).

The decreasing numbers, from the base upwards, of the *Pitymys arvalidens* and the greatly increasing numbers of the *Microtus conjungens*—an intermediate form between *Microtus hintoni-gregaloides* and *Microtus gregalis*—are a clear indication of a deteriorating climate during the laying down of the upper layers of the site.

M. Kretzoi
L. Vértes

Hungarian National Museum, Budapest.

[1] Kretzoi, M., and Vértes, L., *Current Anthropology*, **6**, No. 1, 74 (1965).

Cranial Capacity of the Hominine from Olduvai Bed I

Prof. P. V. Tobias[1] has produced a range of values for the cranial capacity of the 'pre-*Zinjanthropus*' juvenile from Bed I, at the *F.L.K.N.N.* I site at Olduvai Gorge, Tanganyika. This communication presents evidence which confirms Tobias's calculations.

The figures which Tobias calculated on the basis of his '*B*/*A*' ratio, the parietal-to-total volume, were 642·7–723·6 c.c. These figures and the method of obtaining them have been criticized[2], and it is generally held that his figures are questionable. The main reason for this unwillingness to accept Tobias's figures rests with his method of reconstruction of a partial endocast from the parietal fragments. Pilbeam and Simons[2] hold that the articulation of the parietals along the sagittal suture would make a considerable difference in the parietal volume, since a more acute angle of articulation along the suture would reduce the width at the base, thereby decreasing the volume.

Since the fragments are from both sides of the cranium, accurate reconstruction is possible by mirroring one side with the other. Thus, the reconstruction should be reasonably correct, disregarding for the present the question of articulation. Assuming also that the range of values found for partial endocasts, parietal-to-total, is within the values obtained (50·22–56·54 per cent), the question of articulation requires consideration.

Using the photographs and scale provided by Tobias[1], a full-scale drawing was made of the endocast both as seen from above and behind. Using the maximal breadth and height as a plane, a template was constructed of the outline of the endocast in this plane, from one side. By simply turning the template over, the mirror image could be traced. Using the most superior point in the mid-sagittal plane as a pivot point, the template was rotated a number of times, and outlines traced on fine-quality tracing paper. A 'reference' outline was made to conform with the scale of the published photograph. The maximum breadth in the coronal plane was varied from the 'reference' value in both directions. In other words, the angle of articulation at the sagittal suture was made both more and less acute. Each outline was traced, giving a total of four complete outlines: (1) at scale; (2) minus 0·5 cm each side; (3) minus 1·0 each side; (4) plus 0·5 cm each side. Thus, maximum breadth was varied from plus 1·0 cm to minus 2·0 cm, a range surely greater than necessary.

A planimeter was then used to obtain the areas enclosed by each of the four outlines, five readings being

taken for each outline. Each was then calculated in terms of the percentage of the 'reference' or scale area. The values resulting are as follows (per cent): (1) 'scale', 100; (2) minus 0·5 cm, 98·3; (3) minus 1·0 cm, 95·9; (4) plus 0·5 cm, 101.

It is immediately apparent that variations in the articulation angle along the suture do not radically reduce volume estimates. In varying the articulation angle, the decrease essentially affects but one dimension, that is, the lateral breadth. At the same time, the vertical dimension is increased. Since the shift is in one direction, it can be seen that by taking some small volume in the coronal plane, $A(dx)$ and integrating from anterior to posterior dimensions, one is essentially adding small volumes until total parietal volume results. What the planimeter method shows is that the loss in area from constriction of the breadth is almost compensated by the increase in depth. The area remains essentially constant, and since the length of the endocast is not affected by constriction the volume remains essentially constant also. It should be appreciated, however, that this method can only give an approximation, since the articulation in question is along a curved surface. If the entire suture were present in the fragments, it would not be possible to vary greatly the angle of articulation. Since the fragments are partial in this respect, some degree of freedom is allowed for articulation, and taking the area of but one plane of coronal orientation at the point of greatest depth would be roughly representative of a series of areas in different coronal planes.

In sum, Tobias's[1] values are probably correct, provided that: (1) the reconstruction is valid; (2) the partial endocast variation, 'B/A' ratio, is within the limits obtained.

It must be added, however, that this confirmation of Tobias's figures should in no way be taken as supporting his conclusions regarding the creation of a new species of early hominid. The parameter of cranial capacity is quite variable in the higher primates. The end-points of the greater than 1,000 c.c. variation between members of modern *Homo* are not placed in separate species, nor are gorillas, the volumes of which vary between 752 c.c. and 320 c.c. (ref. 3). Thus, it would not be unlikely that on the basis of cranial capacity, at least, the 'pre-*Zinj*' materials are within the Australopithecine taxon of early hominids.

Ralph L. Holloway, jun.

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Columbia University, New York.

[1] Tobias, P. V., *Nature*, **202**, 3 (1964).
[2] Pilbeam, D. R., and Simons, E. L., *American Sci.*, **53**, 237 (1965).
[3] Schultz, A. H., *Anthropol. Anz.*, **25**, 197 (1962).

Prof. Holloway has shown effectively that the partial endocast of *Homo habilis* provides a valid basis for estimating its cranial capacity, and even for a far greater range of angles along the sagittal suture than are anatomically likely. He has increased the maximum (biasterionic) breadth by as much as 10 mm and reduced it by as much as 20 mm and found a minimal effect on the cross-sectional area of the biparietal tunnel. It is of interest that the difference in biasterionic breadth between two reconstructions made in Nairobi and in Johannesburg was scarcely 2 mm. The first was made by Dr. L. S. B. Leakey and myself, with the auxiliary observational judgment of Dr. G. G. Simpson, Mrs. M. Leakey and Mrs. S. Coryndon; the second was made by A. R. Hughes and myself. The tolerance limits of our reconstructions were 2 mm; Prof. Holloway has validated the method for a range of 30 mm between the greatest and smallest biasterionic distances!

Two comments may be added on variability in higher primates:

(1) The capacity of 680 c.c. in *H. habilis* exceeds the australopithecine mean of 502 c.c. ($n = 7$) by 3·79 standard deviations. (The *S.D.* of 47·0 c.c. is an estimate of the population *S.D.*, based on an australopithecine sample range of 435–562 c.c. For this calculation, the adult capacity of the Taung fossil was re-computed from the newer growth data of Ashton and Spence[1], which showed that, by the dental age of the Taung child, the cranial capacities of hominoids have reached 90–94 per cent of the adult mean values. The proposed new 'adult value' for Taung of 562 c.c. replaces the 600 c.c. I formerly accepted[2]. Even on the former estimate, however, 680 c.c. was 3 *S.D.*'s higher than the australopithecine mean.)

(2) It is extremely hazardous to apply to a fossil group such as *Australopithecus* the *a priori* argument that its variability must have been at least of the order found in living higher primates. There is much evidence that the variability of metrical characters may vary from taxon to taxon; thus, for cranial capacity, the coefficient of variation may be as low as 6·8 in chimpanzee and as high as 13·6 in gorilla (♂+♀), or 12·8 in modern man. Even if we separate male and female gorilla capacities, the gorilla male *V* of 10·4 is higher than that of orang-utan or chimpanzee for both sexes combined! Many factors enhance the variance and these may not operate equally in different hominoids, for example, varying degrees of sexual dimorphism, the possible effect of zoo conditions on higher primates and the proportions of zoo-reared and wild-shot animals represented in a skeletal series, the degree of polytypism and polymorphism within a taxon, etc.

Not only may a single metrical character vary to different degrees from population to population, but also different taxa may show high or low overall variability. Morant[3] proved that, in contrast with modern man, Neandertal man was remarkably homogeneous in craniometric characters. This was stressed again not long ago by Blanc, by Vallois and by Caspari[4], the latter adding that "in recent man interindividual variability appears to be relatively large as compared with a number of other species". The Biometric School produced many investigations which showed wide diversity of variability among different human populations and different great apes.

We simply do not know if *Australopithecus* was a highly variable taxon in general, or whether cranial capacity in this group was of low or high variability. The estimated coefficient of variation based on the present australopithecine sample of 7 cranial capacities is 9·35 per cent, which is higher than that of smaller-brained apes, but lower than that of gorilla and modern man. But the problems intrinsic in palaeontological sampling make it very likely that this is an imperfect estimate. Further specimens of australopithecines may place the value of 680 c.c. well within the mean +3 *S.D.*'s; but we cannot assume that this will be so, nor can we base statements on the possible features of specimens still to be discovered. On the existing sample, the estimated cranial capacity of *H. habilis* is significantly greater than the mean australopithecine capacity.

Note added in proof. Dr. J. K. Woo[5] has meanwhile applied my method to his important new pithecanthropine cranium from Lantian, Shensi Province. From a biparietal reconstruction and endocast, he has obtained estimates of the total endocast volume of 775–783 c.c. Using one of Pearson's formulae on the complete reconstructed cranium, he has obtained a value of 778 c.c. These remarkably similar estimates agree with Woo's total morphological assessment in aligning the Lantian specimen with the small-brained Indonesian crania of *Homo erectus*.

P. V. Tobias

University of the Witwatersrand,
Johannesburg.

[1] Ashton, E. H., and Spence, T. F., *Proc. Zool. Soc. Lond.*, **130**, 169 (1958).
[2] Tobias, P. V., *Nature*, **197**, 743 (1963).
[3] Morant, G. M., *Ann. Eugen.*, **2**, 318 (1927).
[4] Caspari, E. W., in *Social Life of Early Man* (Chicago, Aldine, 1961).
[5] Woo, J. K., *Scientia Sinica*, **14**, 1032 (1965).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 11

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (Joint Conference with the Schools Council, at 1 Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m.—Conference on "Applied Science and Engineering Activities in Schools".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. B. W. Burt: "Surface-Active Agents in the Pharmaceutical Field".

UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Drews (University of Freiburg): "The Cell of the Photosynthetic Bacteria in Light- and Dark-Metabolism" (further lecture on October 13).*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Germany: a Regional Geography" (colour film).

Tuesday, October 12

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. H. E. Lewis: "The Islanders of Tristan da Cunha—a Medical Study of Isolation".*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. E. M. Wilson: "A Study of the Feasibility of Tidal Power from Loughs Strangford and Carlingford with Pumped Storage at Rostrevor".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Annual Meeting. 6.30 p.m.—Mr. E. S. Moult: "An Engine Designer's Scrapbook" (Chairman's Address).

Wednesday, October 13

SCHOOL OF PHARMACY, UNIVERSITY OF LONDON (in the Assembly Hall, Brunswick Square, London, W.C.1), at 3 p.m.—The Lord Brain, Bt., D.M., F.R.C.P., F.R.S.: "Drug Dependence".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Mr. M. Hills: "Allocation Rules and Their Error Rates".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. D. C. Cooper: "Signal Processing Using Optical Techniques".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "The Future of Food Legislation". Mr. D. D. Moir: "The Public Analyst's View"; Dr. A. M. Taylor: "The Viewpoint of the Manufacturer".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Electrical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6.30 p.m.—Prof. C. Cherry: "The Communication Explosion".

Thursday, October 14

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUPS (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. Marsh: "The Industrial Training Act".

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Motorist's View of Road Problems", introduced by Mr. E. Shipley.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. E. S. Mathias: "Long Wavelength Laser Generation".

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Dirt in Fluid Power Systems—Should it be Excluded or Tolerated?"

Friday, October 15

ARTHUR STANLEY EDDINGTON MEMORIAL TRUST (in Room 3, Mill Lane Lecture Rooms, The University, Cambridge), at 5 p.m.—Sir John C. Eccles, F.R.S.: "The Brain and the Unity of Conscious Experience" (1965 Eddington Memorial Lecture).

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Flow Visualization".

Saturday, October 16

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. John Okell: "Burmese Musical Instruments".*

Monday, October 18

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. A. Gill: "Impact Testing and Brittle Failure".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. B. A. D. Stocker: "Salmonella Antigens—Structure and Genetics" (further lecture on October 25).*

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Prof. D. B. Spalding: "Heat Transfer Aspects of Coal Utilization" (Fourteenth Coal Science Lecture).

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. H. Hamence: "The Public Analyst's Approach to Pesticide Residues".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Whither Small V-Form Diesel Engines?"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Charles Swithinbank: "A Year with the Russians in Antarctica".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

POSTDOCTORAL FELLOW (cell biologist or biochemist) IN THE DEPARTMENT OF ZOOLOGY to work with Dr. L. G. E. Bell on problems associated with cell surface function—The Deputy Secretary, The University, Southampton (October 12).

LECTURER (with a good honours degree in a science subject and preferably experience of teaching chemistry in secondary schools; a knowledge of Welsh will be an additional qualification) IN EDUCATION—The Registrar, University College of North Wales, Bangor, North Wales (October 14).

LECTURERS (with a good honours degree and a keen interest in any branch of electronics and control engineering) IN THE ELECTRONICS AND CONTROL DIVISION, School of Engineering Science—The Registrar, University College of North Wales, Bangor, North Wales (October 14).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in analysis) IN PURE MATHEMATICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (October 18).

LECTURER IN METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (October 18).

ASSISTANT LECTURER (with a wide interest in genetics so as to be able to lecture to students of both departments) IN GENETICS IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 174/65/Na (October 20).

LECTURER (Grade II) IN THEORETICAL CHEMISTRY—The Registrar, University Senate House, Bristol, 2 (October 20).

LECTURER or ASSISTANT LECTURER (with research experience in any branch of computer science and preferably an interest in programming languages, systems programming or non-numerical applications of computers) IN THE COMPUTATION LABORATORY, DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University, Southampton (October 22).

RESEARCH FELLOW (with a first- or second-class honours degree, and preferably ecological training and previous research experience) IN THE DEPARTMENT OF ZOOLOGY, to work on the general ecology of the fox for a period of three years—The Secretary, The University, Aberdeen (October 23).

SENIOR LABORATORY ADMINISTRATOR (with previous administrative experience, and preferably laboratory or medical knowledge) IN THE DEPARTMENT OF ANATOMY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (October 23).

CHAIR OF MATHEMATICS and HEAD OF THE DEPARTMENT OF MATHEMATICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (October 25).

CHAIR OF NUMERICAL ANALYSIS—The Registrar, University of Strathclyde, Glasgow, C.1 (October 25).

LECTURERS IN NUMERICAL ANALYSIS; LECTURERS IN STATISTICS; and a LECTURER and an ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, University of Strathclyde, Glasgow, C.1 (October 25).

RESEARCH ASSISTANT (male or female honours graduate in any of the biological sciences, including medicine, interested in animal cell biology, and preferably laboratory experience in culture techniques) IN TISSUE CULTURE IN THE DEPARTMENT OF ANATOMY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (October 25).

WELLCOME CHAIR OF PHARMACOLOGY AT THE SCHOOL OF PHARMACY—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 28).

SENIOR LECTURER IN PHYSICS AND THEORETICAL PHYSICS; a SENIOR LECTURER IN HIGHWAY ENGINEERING; a SENIOR LECTURER/LECTURER IN ANTHROPOLOGY; a LECTURER or SENIOR LECTURER IN GEOPHYSICS, a LECTURER IN MICROBIOLOGY; and a SENIOR DEMONSTRATOR/DEMONSTRATOR IN ANTHROPOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 29).

SENIOR LECTURERS or LECTURERS (3) IN GEOLOGY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 29).

ASSISTANT CURATOR (with field experience in ethnology) OF THE MUSEUM OF ARCHAEOLOGY AND ETHNOLOGY—The Secretary of the Appointments Committee, Faculty of Archaeology and Anthropology, University of Cambridge, Downing Street, Cambridge (October 30).

CHAIR OF BOTANY—The Deputy Secretary, The University, Southampton (October 30).

EXPERIMENTAL OFFICER (young graduate interested in the design and construction of electronic and electro-acoustic equipment) IN THE DEPARTMENT OF AUDIOLOGY AND EDUCATION OF THE DEAF—The Registrar, The University, Manchester, 13, quoting Ref. 179/65/Na (October 30).

LECTURER IN THE DEPARTMENT OF PHARMACOLOGY—The Clerk to the Council, School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1 (October 30).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 31).

SENIOR LECTURER or LECTURER IN AGRICULTURAL ENGINEERING at the University of Khartoum—The Registrar, University of Khartoum, c/o The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 31).

LECTURER (preferably with experience in comparative and cellular physiology) IN THE DEPARTMENT OF ZOOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 1).

RESEARCH STUDENT IN METALLURGY for work concerned with the mass-transfer of alloying elements, especially carbon, between plain carbon and alloy steels, immersed in high purity molten sodium at temperatures up to 850 deg. C.—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 1).

CHAIR OF PLANT SCIENCE; and LECTURERS IN BIOLOGICAL SCIENCE at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 4).

DEPARTMENTAL ADMINISTRATOR IN THE DEPARTMENT OF CHEMISTRY—The Deputy Secretary, The University, Southampton (November 6).

SENIOR LECTURER or LECTURER (medically qualified with adequate experience) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (November 8).

SENIOR LECTURER or LECTURER (chemical engineer, preferably with industrial experience of process control) IN CHEMICAL PROCESS CONTROL IN THE DEPARTMENT OF SYSTEMS ENGINEERING—The Secretary, University of Lancaster, Bailrigg House, Lancaster (November 13).

PRINCIPAL OF THE WEST OF SCOTLAND AGRICULTURAL COLLEGE and PROFESSOR OF AGRICULTURE in the University of Glasgow (combined appointment)—The Principal, The West of Scotland Agricultural College, 6, Blythswood Square, Glasgow, C.2 (November 15).

POST-DOCTORAL FELLOW (chemist, biologist or physicist with experience in electron microscopy, with a Ph.D. degree or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability) IN ELECTRON MICROSCOPY IN THE DIVISION OF PROTEIN CHEMISTRY, Commonwealth Scientific and Industrial Research Organization, Parkville, Melbourne—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 462/236 (November 20).

ASSISTANT BACTERIOLOGIST (preferably female, with a university degree and/or practical experience in food microbiology)—The Director, Research Station, The Fruit and Vegetable Canning and Quick Freezing Research Association, Chipping Campden, Glos.

ASSISTANT LECTURER or LECTURER (with research interests in nuclear magnetic resonance spectroscopy) IN ORGANIC CHEMISTRY—Prof. D. H. R. Barton, F.R.S., Department of Chemistry, Imperial College of Science and Technology, London, S.W.7.

ASSISTANT LECTURER (with appropriate qualifications in biology, mycology or botany, and preferably special qualifications in plant mycology or general mycology) IN BIOLOGY—The Registrar, University of Strathclyde, Glasgow, C.1.

CHEMISTRY MASTER (well qualified) to teach up to advanced level—The Headmaster, Latymer Upper School, Hammersmith, London, W.6.

DEMONSTRATOR (honours B.Sc. biochemistry or chemistry) TO ASSIST IN LABORATORY AND TUTORIAL INSTRUCTION IN THE DEPARTMENT OF BIOCHEMISTRY—The Secretary, Royal Free Hospital, School of Medicine (University of London), Hunter Street, London, W.C.1.

ELECTRON MICROSCOPE TECHNICIAN (with experience in biological and physical work and a sound knowledge of replica, embedding and sectioning techniques) to take charge of the operation and maintenance of a Siemens Elmiskop MKI and ancillary equipment—The Director of Research, Natural Rubber Producers' Research Association, 56 Tewin Road, Welwyn Garden City, Herts.

ENTOMOLOGIST (with a B.Sc. degree with honours in zoology or entomology, and preferably practical experience of control of field pests and pests of stored products) in British Honduras, to advise the Agricultural Extension Staff and farmers on pest control measures, to evaluate insecticides and undertake basic field research in pest control, to lecture on pests and pest control and to advise on legislation to control the use of certain insecticides, and pest control generally—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/26/03.

EXPERIMENTAL OFFICER to work on electronic instrumentation in the field of cardiology—The House Governor, Charing Cross Hospital, London, W.C.2.

HEAD OF THE NEW ZEALAND SCHOOL OF PHARMACY, Central Institute of Technology—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, or The Director of Education, Private Bag, Wellington, New Zealand.

MICROANALYST (with experience in micro-elementary and functional group analysis, and preferably familiarity with the operation and maintenance of electronic equipment) IN THE ORGANIC CHEMISTRY DIVISION of the National Chemical Research Laboratory, C.S.I.R., South Africa, to take charge of the routine microanalytical operations of the laboratory—The South African Scientific Liaison Officer, Chichester House, 278 High Holborn, London, W.C.1.

POST-DOCTORAL ASSISTANT; and a TECHNICAL ASSISTANT IN INORGANIC CHEMISTRY to work under the supervision of Dr. J. F. Nixon—The Secretary, Department of Chemistry, St. Salvator's College, University of St. Andrews, St. Andrews, Fife, Scotland.

PRINCIPAL GRADE BIOCHEMIST (with a higher degree in chemistry or biochemistry)—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. No. PT/836.

RESEARCH ASSISTANT (preferably with a degree in botany, agriculture, agricultural botany or other equivalent qualification) IN THE POTATO BRANCH for work on herbicides in relation to potato varieties—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

RESEARCH ASSISTANT (zoology graduate) IN THE DEPARTMENT OF ZOOLOGY AND APPLIED ENTOMOLOGY, Imperial College of Science and Technology, to work at the College Field Station, Silwood Park, on chemical factors in host plant selection by phytophagous insects or on the reproductive behaviour of insects—Dr. C. T. Lewis, Silwood Park, Sunninghill, Ascot, Berks.

RESEARCH ASSISTANTS (with a degree in chemistry or equivalent and preferably interested in the kinetics of exchange reactions) IN RADIOCHEMICAL STUDIES—The Senior Lecturer in Radiochemistry, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH ASSISTANTS (with a good degree in physics or electrical engineering) for work on resonances excited by microwaves in the plasma of a discharge column and on the application of these resonances in the field of electronics—Prof. M. Potok, Royal Military College of Science, Shrivenham, near Swindon, Wiltshire.

RESEARCH ASSISTANTS (2) (with qualifications in engineering or physics and preferably some experience in research) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, for work in the fields of (*a*) hydrodynamic lubrication, and (*b*) fluid control systems—The Registrar, The University, Sheffield, 10.

SENIOR TECHNICIAN IN THE DEPARTMENT OF CHEMICAL PATHOLOGY to assist in the investigation of sick children—The Chemical Pathologist, Hospital for Sick Children, Institute of Child Health, Great Ormond Street, London, W.C.1.

SENIOR TECHNICIAN (preferably with previous experience with polymer equipment) for the maintenance and operation of modern equipment in a polymer characterization laboratory—The Departmental Superintendent, Chemistry Department, Imperial College, London, S.W.7.

SENIOR TECHNICIAN (with H.N.C. or equivalent qualification in physics, chemistry or biology, and preferably experience in physical instrumentation and an interest in field work) IN THE ECOLOGY SECTION OF THE DEPARTMENT OF FORESTRY—Dr. M. E. D. Poore, Department of Forestry, Forestry Institute, University of Oxford, Oxford.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 16, No. 2: A Systematic Revision of the Ameniinae (Diptera: Calliphoridae). By R. W. Crosskey. Pp. 33–140. 35*s*. Vol. 16, No. 3: A Revision of the Nodini and a Key to the Genera of Eumolpidae of Africa (Coleoptera: Eumolpidae). By B. J. Selman. Pp. 141–174. 14*s*. (London: British Museum (Natural History), 1965.) [268

Water Resources Board. Logarithmic Plotting of Stage-discharge Observations. By A. I. G. S. Robertson. Pp. 36. (T.N.3.) Originally published 1962. (Reading: Water Resources Board, 1965.) [268

Philosophical Transactions of The Royal Society of London. Series B: Biological Sciences. No. 758, Vol. 249 (26 August, 1965): A Submerged Late-quaternary Deposit at Roddans Port on the North-East Coast of Ireland. By M. E. S. Morrison and N. Stephens. (With Appendixes by Mrs. Margaret Jope and S. P. Dance, F. W. Anderson, F. E. Round and H. Godwin, and E. H. Willis.) Pp. 221–225. (London: The Royal Society, 1965.) 18*s*.; 2.70 dollars. [268

University of Bristol. Annual Report of the Long Ashton Agricultural and Horticultural Research Station (The National Fruit and Cider Institute) for the year 1964. Pp. xii + 229 + 5 plates. (Long Ashton: The Agricultural and Horticultural Research Station, 1965.) 15*s*. [268

Fourth Report from the Select Committee on Procedure, together with the Proceedings of the Committee Relating to the Report, Minutes of Evidence and Appendices, Session 1964–65. Pp. xxiii + 148. (London: H.M. Stationery Office, 1965.) 15*s*. net. [39

Other Countries

Republic of Zambia: Ministry of Lands and Natural Resources. Annual Report of the Department of Water Affairs for the year 1964. Pp. 9. (Lusaka: Government Printer, 1965.) 2*s*. [268

The Vegetation of Uganda and Its Bearing on Land-use. By I. Langdale-Brown, H. A. Osmaston and J. G. Wilson. Pp. 159 + 24 plates. (Entebbe: Government Printer; London: The Uganda High Commission, 1964.) Shs. 30/–. [268

Académie Royale de Belgique. Classe des Sciences—Mémoires. Tome 34, Fascicule 1: On the Summability Methods of Divergent Series. By Kanuo Ishiguro. Pp. 43. 60 francs. Tome 35, Fascicule 3: Schistosité et Granitisation dans le Synclionorium de Laval et ses Abords. Par Paul Fourmarier, Annik Palhate et Bernard Auvray. Pp. 60. 140 francs. Tome 35, Fascicule 4a et 4b: Étude Expérimentale et Interprétation du Spectre Infra-Rouge des Silicates et des Germanates. Application à des Problèmes Structuraux Relatifs à l'état Solide. Fascicule 1: Pp. 261. Fascicule 2: Pp. 134 (164 fig.). 540 francs. (Bruxelles: Académie Royale de Belgique, 1965.) [149

Records of the Australian Museum. Vol. 26, No. 7 (12th June, 1964): Soil and Landscape History in the Vicinity of Archaeological Sites at Glen Davis, New South Wales. By P. H. Walker Pp. 247–264 + plates 25–27. 6*s*. Vol. 26, No. 8 (19th June, 1964): Notes on the Genera *Amoria* Gray, 1855 and *Zebramoria* Iredale, 1924 (Gastropoda: Volutidae), with Descriptions of New Species. By Donald F. McMichael. Pp. 265–274 + plate 28. 3*s*. Vol. 26, No. 9 (28th June, 1964): Rediscovery of the Echinod *Clypeaster tumidus* (Tenison-Woods) and an Amended Description. By R. Endean and Elizabeth Pope. Pp. 275–281 + plates 29 and 30. 2*s*. 6*d*. Vol. 26, No. 10 (17th July, 1964): New Linguloid Shells from Lower Ordovician and Middle Palaeozoic Rocks of New South Wales. By H. O. Fletcher. Pp. 283–294 + plates 31 and 32. 4*s*. Vol. 26, No. 13 (30th December, 1964): Merostomoidea (Arthropoda, Trilobitomorpha) from the Australian Middle Triassic. By E. F. Riek. Pp. 327–332 + plate 35. 2*s*. (Sydney: The Australian Museum, 1964.) [149

New Zealand Forest Service: Forest Research Institute. Research Notes, No. 41: Trials with Arboricides for Control of Coppice Growth of Indigenous Hardwoods. By A. E. Beveridge. Pp. 10. (Wellington: Government Printer, 1965.) [149

Mutations in Quantitative Traits: Report on Symposium held on July 28 and 29, 1964. (Gamma Field Symposia, No. 3.) Pp. iii + 150. (Ohmiya-machi, Ibaraki-Ken, Japan: The Institute of Radiation Breeding, Ministry of Agriculture and Forestry, 1965.) [149

Académie Royale de Belgique. Classe des Sciences—Mémoires. Tome 35, Fascicule 5: A Study of Molecular and Physical Processes in Comets. By Dr. Claude Arpigny. Pp. 157. (Bruxelles: Académie Royale de Belgique, 1965.) 200 francs. [149

Western Australian Naturalists' Club. Handbook No. 8: Frogs of Southern Western Australia. By A. R. Main. Pp. 73 (5 plates). (Perth: Western Australian Naturalists' Club, 1965.) A6*s*. [149

National Hydatids Council, Wellington, New Zealand. Fifth Annual Report and Statement of Accounts for the year ended 31st March, 1965. Pp. 40. Information Bulletin No. 15: Hydatids Eradication in Iceland. Pp. 11. Hydatid Disease in New Zealand: An Account of the Events leading to the Establishment of the National Hydatids Council, and the Methods adopted to Eradicate the Disease. By F. S. Maclean. Pp. 48. Parasitic Animals with Special Reference to Hydatid Disease and Its Prevention. By R. M. Miller.. (Reprinted from "Biology for Sixth Forms". Teach and Test Publications, Ltd., Otahuhu, New Zealand.) Pp. 36. (Wellington: The National Hydatids Council, 1965.) [149

Annals of the New York Academy of Sciences. Vol. 123, Article 2: Geological Problems in Lunar Research. By J. E. M. Adler and 60 other authors. Pp. 367–1257. (New York: New York Academy of Sciences, 1965.) [149

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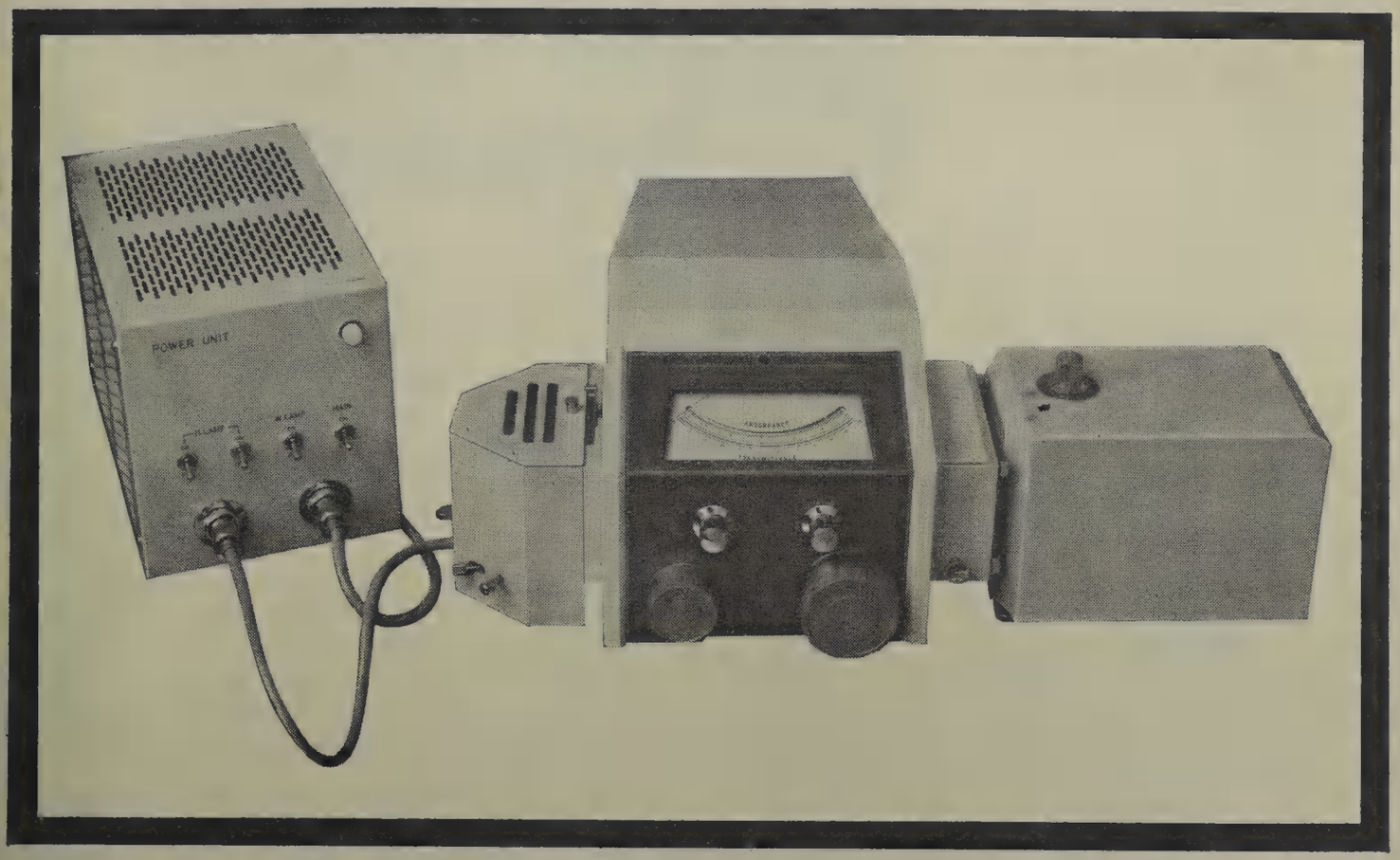

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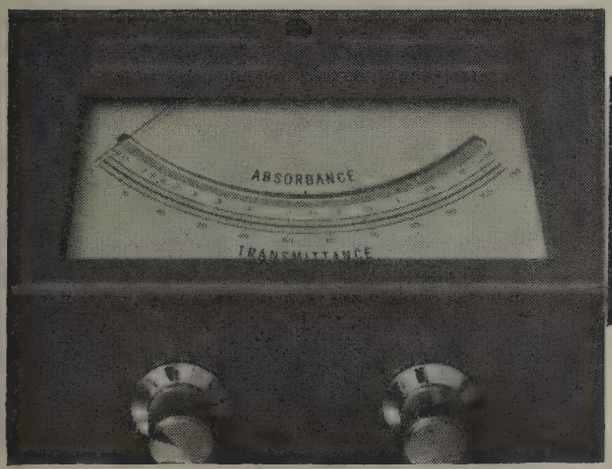

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Geologist:
Geological investigations in the exploration and development of mineral resources, regional geological mapping and engineering geology.

Qualifications:
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Applicants should possess a University Degree preferably with Honours, including Geology as a major subject, and should have at least seven years' experience in the appropriate field.
Geologist:
Applicants should possess a University Degree preferably with Honours, including Geology as a major subject.

Passages from the United Kingdom are provided under the Commonwealth Migration Scheme, arranged through the Agent-General for Tasmania.
Recreation leave, sick leave, and long service leave are provided.
Superannuation is compulsory for permanent officers.

Applications, in duplicate, should be addressed to the Agent-General for Tasmania, 457 Strand, London, W.C.2.

CHIEF TECHNICAL ADVISER —AGRICULTURE: £2,000 per annum

This Company produces limes and fertilizers which it markets directly in the North of England and Scotland through its sales force of 65 salaried representatives.

The principal duties are:
(a) The technical training of the sales force in fertilizer usage.
(b) Supervision of the Company's laboratory (product and soil analysis).
(c) The annual conduct of field trials of fertilizers.
(d) Advising the Board on new fertilizer practices.

Applicants must have degrees in Agriculture. Preference will be given to those with roughly parallel experience in commercial organizations.
Car provided; Pension Scheme; all expenses paid.
Brief applications, outlining qualifications and experience to:

Managing Director
ADAM LYTHGOE LIMITED,
CULCHETH, WARRINGTON,
Lancashire.

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a Post-doctoral Research Biochemist, to join group engaged in studying the control of protein Biosynthesis in animal cells. Salary within the range of £1,400 to £1,600.

Applications to Professor H. R. V. Arnstein, Department of Biochemistry, University of London, King's College, Strand, London, W.C.2.

WESTMINSTER HOSPITAL TEACHING GROUP

QUEEN MARY'S HOSPITAL
ROEHAMPTON, S.W.15

Senior Biochemist required to take charge of routine Chemical Pathology Department. Opportunities for research. Queen Mary's Hospital is now a general hospital of over 400 beds, dealing with acute medical and surgical cases.

Applications, stating age, qualifications and names of two referees to Hospital Secretary within 21 days of publication.

MYCOLOGIST

required by

AN FORAS TALUNTAIS

(The Agricultural Institute)

in its Soil Biology Department. Appointment up to Senior Research Officer level depending on qualifications and experience. Marriage and children's allowances.

Superannuation Scheme including special arrangement for F.S.S.U. member.

ESSENTIAL: Honours University Degree in Science or equivalent.

DESIRABLE: Suitable research experience.

Application forms and further particulars from the Director, An Foras Taluntais, 33 Merrion Road, Dublin 4. Latest date for receipt of completed forms, November 4, 1965.

THE UNITED STEEL COMPANIES LIMITED
RESEARCH AND DEVELOPMENT DEPARTMENT

DEPUTY LIBRARIAN

A deputy is required for the large industrial and scientific library of the Research and Development Department of The United Steel Companies.

An Associate of the Library Association with a professional knowledge of cataloguing and reference work in science and technology would be preferred. Candidates with partial library qualifications will be considered provided that they have had suitable experience.

Co-ordinate indexing has been introduced recently and there is scope for the exercise of personal initiative in developing the library services. The work will include supervision of the library's clerical staff.

Competitive salary. Excellent working conditions in pleasant surroundings. 5-day week. Contributory pension scheme. Excellent catering. Private transport between Sheffield and the laboratories.

APPLY TO: **THE DEPUTY DIRECTOR OF RESEARCH**
THE UNITED STEEL COMPANIES LIMITED
RESEARCH AND DEVELOPMENT DEPARTMENT
SWINDEN LABORATORIES, MOORGATE, ROTHERHAM.

PUBLIC SERVICE OF TASMANIA, AUSTRALIA

CERAMIC TECHNOLOGIST

DEPARTMENT OF MINES

Location : Launceston.

Applications are invited for appointment to a position of Ceramic Technologist in the Department of Mines, Tasmania.

Salary : Class V : £A1,442 per annum then £A1,540 by £A112 to £A1,988 per annum.
Class IV : £A2,100 by £A112 to £A2,324 per annum.
Class III :
Grade 2 : £A2,436 by £A112 to £A2,548 per annum.
Grade 1 : £A2,625 by £A112 to £A2,737 per annum.
These salaries will be increased by approximately 1½ per cent. Precise scales have yet to issue.

Commencing salary will be according to qualifications and years of experience, and will be payable from date of embarkation for Australia.

Duties : The successful applicant will be responsible to the Chief Chemist and Metallurgist and will be required to conduct research work on clay and other raw materials used in the manufacture of bricks, tiles, pipes, etc., and to examine and report upon the finished products. He will also be required to examine production practices in Tasmania and assist manufacturers to improve methods of manufacture.

Qualifications :
University Degree or Technical College Diploma with Chemistry as a major subject, and experience in manufacturing processes and testing of ceramic materials. Preference will be given to applicants who have completed a course in Ceramic Technology.

Passages from the United Kingdom are provided under the Commonwealth Migration Scheme, arranged through the Agent-General for Tasmania.

Recreation leave, sick leave, and long-service leave are provided. Superannuation is compulsory for permanent officers.

Applications, in duplicate, should be addressed to the Agent-General for Tasmania, 457 Strand, London, W.C.2.

LOUGHBOROUGH COLLEGE OF TECHNOLOGY

LOUGHBOROUGH, LEICESTERSHIRE
(University Designate)

Applications are invited for the following additional post in the department of Mechanical Engineering: Reader in Mechanical Engineering: Applicants should possess good academic qualifications, have had industrial experience and have engaged in research in industry or allied fields. They should be prepared to develop research groups. Salary will be paid according to the scales: Reader: £2,550 by £100 to £3,250 per annum.

Further particulars may be obtained from the Academic Registrar. In reply please quote reference 41/AF.

ROYAL HOLLOWAY COLLEGE

UNIVERSITY OF LONDON
DEPARTMENT OF PHYSICS

Applications are invited for the post of Research Assistant (Academic) to assist in an investigation into laser-induced surface damage under the direction of Professor S. Tolansky, F.R.S. The successful applicant will be permitted to study for a higher degree of the University of London. The appointment will be for two years in the first instance: salary in the range of £1,050 by £75 to £1,275.

Four copies of applications, giving full particulars and the names and addresses of two referees, should be sent as soon as possible to the Secretary, Royal Holloway College, Englefield Green, Surrey.

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

DEPARTMENT OF CHEMISTRY

Applications are invited for the post of CHIEF STOREKEEPER in the DEPARTMENT. It is essential that an applicant should have had experience in the supply and maintenance of scientific or technological laboratories. The successful candidate would rank as a Senior Technician on a salary scale which is at present £840 rising by annual increments to £1,040. The position on the scale at which the appointment is made will be determined by nature and length of experience. There is a Pension Scheme.

Applications, giving cognate personal and professional details and accompanied by the names of two referees, should be sent by October 25, 1965, to Professor Stanley Peat, Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire.

HUNTINGDON RESEARCH CENTRE

Due to the continued rapid expansion of this research organization there are now further vacancies for male and female RESEARCH ASSISTANTS and ANIMAL TECHNICIANS in all grades for interesting and varied work in the general field of drug evaluation. Previous experience with animals is an advantage, but this is not essential ■ full training will be given and facilities for further study are available.

Good salaries will be paid to the right applicants and benefits include three weeks holiday annually.

Applications, which will be treated in confidence, should be made in writing to the Administrative Officer, Huntingdon Research Centre, Huntingdon, quoting reference H/N/11/T.

GAS CHROMATOGRAPHY LIMITED

(A Member of the Fisons Group)

Requires

APPLICATIONS MANAGER

A very important post with excellent prospects as a substantial expansion is now planned. Only Graduates with considerable gas chromatography experience, pleasant personality, ability to give lectures and talks and do some original work need apply. Initially the appointment involves both selling and research work in gas chromatography but is expected to expand into other fields later on. Salary level in excess of £1,750 per annum, but dependent on age, qualifications etc.

Write in first instance in confidence to Gas Chromatography Limited, Boyn Valley Road, Maidenhead, Berks.

UNIVERSITY OF LIVERPOOL

Applications are invited for the post of

COMPUTER OFFICER

at the Tidal Institute and Observatory. The salary scale range is £900 to £1,845 per annum. The initial salary will be £900 per annum. Superannuation scheme.

Applicants must possess a suitable University degree and have had experience in computer programming and operation.

Applications, stating age, qualifications and experience, together with the names of two referees, should be received not later than October 23, 1965, by the Registrar, from whom further particulars can be obtained. Please quote Ref. CV/296/N.

UNIVERSITY OF LAGOS MEDICAL SCHOOL

Applications are invited for two Lectureships in Anatomy in Department of Morphological Sciences. Candidates must have medical qualifications; specialist qualification in anatomy or allied subject and research experience desirable. Appointees will be required to teach Human, Gross and Microscopic Anatomy. The department is engaged in research and would support any feasible research of the appointees own choice. Appointments to commence as soon as possible. Salary Scale: £2,200 by £100 to £2,600 per annum. Family passages. Children's and car allowances. Superannuation Scheme. Part-furnished accommodation. Regular U.K. leave.

Detailed applications (six copies), naming three referees by November 2, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF LANCASTER

DEPARTMENT OF SYSTEMS ENGINEERING

Applications are invited for the post of Senior Lecturer or Lecturer in Chemical Process Control. This is the second appointment in a group which will be concerned with the construction of mathematical models for industrial processes and their utilization in process control. The candidate should be a chemical engineer, preferably with industrial experience of process control.

Further particulars of the appointment may be obtained from the Secretary, University of Lancaster, Bailrigg House, Lancaster, to whom applications should be sent not later than November 13, 1965.

THE DISTILLERS COMPANY LIMITED

Research Chemists

The Distillers Company Limited have openings for well qualified Research Chemists (men or women) in the laboratories of the Research Department at Great Burgh, Epsom, Surrey.

The chemists appointed will undertake long-range organic and physico-organic research directed towards the development of novel processes for the large-scale manufacture of industrial organic chemicals from petroleum sources, and within broad limits of policy they will enjoy full freedom to exercise their talents for research.

Applicants, preferably aged under 30, should be organic or physical chemists with wide ranging interests. Scientific initiative and imagination based on a sound theoretical knowledge of chemistry at a good honours degree standard is essential; experience of academic or industrial research is desirable, but applications will be welcomed from candidates who have recently qualified or will shortly do so.

The Company operates a non-contributory pension scheme; assistance can be given to married men towards house purchase and removal expenses.

Please send brief details, quoting Ref. No. 5/126 to:

Staff Manager,
THE DISTILLERS COMPANY LIMITED,
(Chemicals & Plastics Group),
21-22 Bolton Street,
London, W.1.

NORTH STAFFORDSHIRE HOSPITAL MANAGEMENT COMMITTEE

NORTH STAFFORDSHIRE ROYAL INFIRMARY (511 BEDS), STOKE-ON-TRENT

Senior Laboratory Technician, Grade I or II, according to qualifications and experience, required in Laboratory of newly formed Metabolic Unit. Applicant should have wide experience in Biochemistry but Final Diploma of I.M.L.T. in Biochemistry not essential. Whitley Council salary scales and conditions of service.

Applications, with names of two referees, to Hospital Secretary, from whom full details may be obtained.

Research and Development in Pharmaceuticals

The Pharmaceuticals Division of I.C.I. Ltd. is looking for a first-class team leader for research and development in pharmaceutics.

The man appointed must not only be a pharmacist of high standing in his profession, but also a leader who has already proved his ability to inspire and manage a team of research and development scientists either in industry or in academic fields.

The team he will lead is responsible for the development of formulations of the Company's products and for research on the relation between formulation and the effectiveness of drugs.

The starting salary and prospects will be in keeping with the responsibilities of this appointment, and, for the right man, there is a clear promotion route to Senior Management in the Division.

Applications, initially with brief details of qualifications and experience should be addressed to:

The Development Director,
IMPERIAL CHEMICAL INDUSTRIES LIMITED,
PHARMACEUTICALS DIVISION,
Fulshaw Hall, WILMSLOW, Cheshire.

COMMONWEALTH OF AUSTRALIA

DEPARTMENT OF HEALTH

NATIONAL BIOLOGICAL STANDARDS LABORATORY, CANBERRA

BACTERIOLOGIST, CLASS 2

Bacterial Products Section

SALARY: £A2,450 to £A2,765 per annum.

DUTIES: Supervise and participate in the testing of bacterial vaccines and carry out investigations into the immunology of vaccination.

QUALIFICATIONS: Bachelor of Science Degree with majors in Microbiology and/or Biochemistry and experience in microbiological laboratory work desirable.

CONDITIONS: First class air/sea transport for the successful applicant and dependants (wife and dependent children). Salary will be paid from date of embarkation.

APPLICATIONS: Further information and application forms can be obtained from:

The Chief Medical Officer, (Ref. B/1)
Australia House, Strand, London, W.C.2.

Closing Date: December 11, 1965.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

DEPARTMENT OF PURE MATHEMATICS

Applications are invited for an Assistant Lecturer or Lecturer in Pure Mathematics. Special interest in Analysis is desirable but not essential.

Applications (six copies), containing the names of three referees, should be received not later than October 29, 1965, by the Registrar, University College, Cardiff, from whom further particulars may be obtained.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

Applications are invited from Zoology graduates for a post of Research Assistant in the Department of Zoology and Applied Entomology. The successful applicant will be required to work at the College Field Station, Silwood Park, on chemical factors in host plant selection by phytophagous insects or on the reproductive behaviour of insects. Salary according to qualifications up to £1,210 per annum, plus F.S.S.U.

Applications should be addressed to Dr. C. T. Lewis, Silwood Park. Sunninghill, Ascot, Berks.

Geigy *The discovery and development of a number of outstandingly successful pharmaceutical preparations used in the treatment of rheumatic and nervous disorders have given Geigy an international reputation in this field.*

RESEARCH CHEMIST

There is a vacancy for a Research Chemist to work on the synthesis of compounds of medicinal interest. The successful candidate will probably be aged 25 to 30 years and will hold a good Honours degree and preferably a higher degree in chemistry. He should already have several years' experience in synthetic organic chemistry which may have been obtained in industry or at an educational institution.

The appointment will be in one of the company's pharmaceutical research laboratories which are situated in a modern, well-equipped laboratory building at one of the company's main locations in Trafford Park, near Manchester.

Geigy (U.K.) Ltd. is a member of a large international group of companies with world-wide interests in the field of fine chemicals and pharmaceuticals. The organisation adopts a very progressive attitude towards the introduction of new techniques and ideas in both management and research and development.

A good competitive salary will be paid, contributory pension scheme and other conditions in keeping with modern practice.

Please write, quoting Ref. G.25/BO, giving brief details of experience and qualifications, to:

Personnel Officer,
Geigy (U.K.) Ltd.,
Simonsway, Manchester, 22.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited from male or female honours graduates in any of the biological sciences including medicine for the post of a Research Assistant in Tissue Culture in the Department of Anatomy, University College, Cardiff. The appointment, for a period of three years, offers opportunities to work for a higher degree. The candidate should be interested in animal cell biology. Laboratory experience, preferably in culture techniques, would be an advantage. The work planned would entail the application of microcinematography, autoradiography and electron microscopy to cultured cells, under the guidance of and in collaboration with Dr. F. Jacoby. Salary £950 per annum. The appointment is subject to the usual terms and conditions of employment of the University College of South Wales and Monmouthshire.

Applications, containing the names and addresses of three referees, should be sent to the Registrar, University College, Cathays Park, Cardiff, and should reach him not later than October 25, 1965.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Experimental Officer in the Department of Audiology and Education of the Deaf. Candidates should be young graduates interested in the design and construction of electronic and electro-acoustic equipment. Salary range: £870 to £1,450 per annum. The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 30, 1965, to the Registrar, the University, Manchester 13, from whom further particulars may be obtained, on quoting reference 179/65/Na.

UNIVERSITY OF BELFAST

DEPARTMENT OF MECHANICAL ENGINEERING

CHAIR OF THERMODYNAMICS AND FLUID MECHANICS

The Senate of The Queen's University of Belfast invites applications for a Chair of Thermodynamics and Fluid Mechanics (a second Chair in the Department of Mechanical Engineering) from April 1, 1966, or such other date as may be arranged. The salary will be £4,000 plus F.S.S.U. Applications should be received by October 30, 1965.

Further particulars may be obtained from G. R. Cowie, M.A., LL.B., J.P., Secretary.

BIOCHEMIST

required by

AN FORAS TALUNTAIS

(The Agricultural Institute)

in its Soil Biology Department for research of legume nitrogen fixation. Appointment up to Senior Research Officer level depending on qualifications and experience. Marriage and children's allowances.

Superannuation scheme including special arrangements for F.S.S.U. member.

ESSENTIAL: Honours University Degree in Science (majoring in biochemistry) or equivalent.

DESIRABLE: Suitable research experience.

Application forms and further particulars from the Director, An Foras Taluntais, 33 Merrion Road, Dublin 4. Latest date for receipt of completed forms, November 4, 1965.

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

SCHOOL OF ENGINEERING SCIENCE

ELECTRONICS AND CONTROL DIVISION

Applications are invited for posts of Lecturers in this rapidly expanding School. Candidates with a good honours degree and a keen research interest in any branch of Electronics and Control Engineering will be considered.

Salary will be according to experience and qualifications on the scale £1,400 by £85 to £2,505. In addition there are F.S.S.U. benefits.

Applications (two copies), giving the names and addresses of two referees, should be sent by November 30, 1965, to the Registrar, University College of North Wales, Bangor, from whom further particulars may be obtained.

UNIVERSITY OF DUBLIN

TRINITY COLLEGE

DEPARTMENT OF PHARMACOLOGY

RESEARCH LECTURER OR SENIOR RESEARCH LECTURER IN MEDICAL STATISTICS: The appointee will be responsible for the statistical design and evaluation of the investigations in Clinical Pharmacology carried out in the Department of Pharmacology and associated hospitals. He will be encouraged to carry out his own research in medical and social statistics. He will be responsible for arranging a course in statistics for medical students in their premedical and clinical curricula, and will be available for giving statistical advice to other Departments in the School of Physic. He will be the representative of the School of Physic in the Department of Mathematics. Salary scale: Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,100; Research Senior Lecturer, £1,850 by £60 to £2,450 per annum.

JUNIOR RESEARCH LECTURER OR RESEARCH LECTURER IN CLINICAL PHARMACOLOGY: This post will be combined with an appointment as Honorary Registrar in the Federated Dublin Hospitals, and will initially be made for a three-year period. Membership of one of the Royal Colleges of Physicians and/or postgraduate experience in clinical research is necessary. Duties will include teaching and research on the clinical applications of drugs and the design and arrangement of therapeutic trials. Salary scale: Junior Research Lecturer, £1,000 by £50 to £1,200 per annum; Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,150 per annum.

JUNIOR RESEARCH LECTURER OR RESEARCH LECTURER IN CLINICAL PSYCHOPHARMACOLOGY: Applications are invited for these posts which will be established in the near future. The post which is filled will be combined with an appointment as Honorary Registrar in St. Patrick's Hospital, Dublin, and will initially be made for a three-year period. A Diploma in Psychological Medicine and/or postgraduate experience in clinical psychiatric research is necessary. Duties will include teaching and research on the clinical applications of drugs and the design and arrangement of therapeutic trials, with special reference to the use of drugs in psychiatric medicine. Salary scale: Junior Research Lecturer, £1,000 by £50 to £1,200 per annum; Research Lecturer, £1,250 to £1,650 (merit bar) by £50 to £2,150.

RESEARCH ASSOCIATE IN CLINICAL PHARMACOLOGY: This post is open for application by students who wish to register for a postgraduate degree in order to carry out research on the clinical applications of drugs in general or psychiatric medicine in hospitals associated with the Department of Pharmacology. Candidates should be medical students of the University of Dublin who have passed their B.A. examination, or should have an Irish Medical degree. Basic salary scale £450 to £500 per annum.

RESEARCH ASSOCIATE IN CHEMICAL PHARMACOLOGY: This post is open for application by candidates who wish to register for the degree of M.Sc. or Ph.D. in order to carry out research on chemical aspects of drug metabolism. Candidates should have an honours degree in organic chemistry or biochemistry. Salary scale £450 to £500 per annum.

Candidates possessing medical qualifications will receive an annual increment of £100 on their basic salary.

For all these appointments (excepting those of Research Associate) starting salaries may be varied according to qualifications and experience. Superannuation contributions are paid by the College, together with marriage and child allowances of £65 per annum and £45 per annum. Further particulars from the Secretary to the College, Trinity College, Dublin, 2. Closing date for applications, October 30, 1965.

ASSISTANT CONSERVATORS OF FORESTS

FIJI

Two Assistant Conservators of Forests are required for Administrative and executive duties in all branches of forestry including the training of local staff. Candidates, must hold a degree in Forestry. Salary, including overseas addition, £1,142 to £2,273 per annum, according to experience plus 15 per cent terminal gratuity. Education and disturbance allowances. Accommodation available. Free medical treatment. Passages provided. 2½- to 3-year contract.

Please apply for further details, giving full name and brief particulars of qualifications and experience, quoting Ref. RC 324/62/03 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

Esso

ESSO RESEARCH LTD

REQUIRE

CHEMISTS, PHYSICISTS and ENGINEERS

for their

RESEARCH CENTRE AT ABINGDON BERKSHIRE

Applicants should have a good honours degree or equivalent, and be not more than 30 years of age. The vacancies are in five broad areas of work, as described below. For all of them a prime requirement is that CREATIVE IMAGINATIVE THINKING which is necessary to forward the aims of a RESEARCH COMPANY.

ADDITIVES RESEARCH involving synthesis, structural and mechanism studies in a wide field of organic and organo metallic chemistry. This work is clearly of interest to organic chemists but physical chemists will also find much to engage them.

ANALYTICAL WORK including the use of instrumental as well as classical chemical techniques in the separation, identification and estimation of structures and compounds. The analytical problems met are frequently complex and the opportunity exists for the use of specialist experience in the development of new techniques.

PETROLEUM PRODUCT QUALITY RESEARCH. The research programmes in this area call for team-work in which CHEMISTS, ENGINEERS and PHYSICISTS work together. A flexible scientific approach is called for, together with the ability to learn and apply appropriate specialist techniques.

MECHANICAL ENGINEERING involving application of mechanical design and thermodynamic principles to the development of experimental and test equipment required in product quality research. Working experience with I/C engines and associated test equipment would be an advantage in these positions.

INSTRUMENTAL AND ELECTRONIC ENGINEERING embracing work on the development of systems for sensing and recording experimental data, and automation of data collection and equipment control, in support of research activities.

Please write, summarizing personal details, qualifications and experience, to:

E.C.G. Jelfs (Ref. 513D),
ESSO RESEARCH LIMITED,
Abingdon, Berkshire.

BROOKE BOND TEA LIMITED
RESEARCH LABORATORIES

BLOUNTS COURT · SONNING COMMON · OXFORDSHIRE

RESEARCH TECHNICIAN

Applications are invited for a Research Technician post in the Company's Research Laboratories. Some previous experience in biochemical, chemical, medical or biological laboratories is necessary. The successful applicant will work with a team of research scientists on problems directed towards investigation of the biochemistry and chemistry of tea.

Starting salary will be within the range £550 to £1,000 per annum depending upon age, qualifications and experience. The Company operates a contributory pension scheme. Application, in duplicate please, to the Head of Research at the above address. The closing date for applications is October 14.

FISONS PHARMACEUTICALS LIMITED

Vacancies exist in the Research Department of FISONS PHARMACEUTICALS LIMITED, at their Holmes Chapel Unit for a

LABORATORY ASSISTANT

to work in the Biochemistry Section.

Applicants for the position should possess at least two Advanced level G.C.E. subjects (one of which is chemistry) or O.N.C. in chemistry.

Successful candidates will be encouraged to achieve further professional qualifications by attending approved Technical College Courses.

The Company is a member of the FISONS GROUP of Companies and Group Contributory Pension and other welfare schemes operate. The company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications, which will be dealt with in confidence, should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

Mitchell Cotts Group Ltd.

require an

EXPERIENCED AGRONOMIST

for service with the Tendaho Plantations Cotton Project in Ethiopia. Candidates will be required to assist the Manager of the Project and must be capable of (a) planning and organising factorial experiments in connection with soil and crop improvements, and (b) carrying out or supervising such field and laboratory testing on the plantation as may be required. Salary according to qualifications and experience. Non-contributory pension scheme. Free furnished housing. Six weeks' home leave on full pay after two years, then similar leave annually.

Applications in confidence to :

Personnel Officer,
MITCHELL COTTS GROUP LTD.,
Cotts House, Camomile Street, London, E.C.3.

DAR-ES-SALAAM TECHNICAL COLLEGE
TANZANIA

Applications are invited for the post of Education Officer to teach Mathematics to engineering students on O.N.C. courses. Candidates should be nationals of the U.K. or the Republic of Ireland, should have a degree in mathematics or H.N.C. in Engineering and experience of teaching mathematics to engineering students. Appointment on contract for two years in the first instance. Salaries: Graduates, £1,281 to £2,280 per annum ; non-graduates, £1,188 to £1,881 per annum. Entry point according to qualifications and experience. Resettlement grant of 25 per cent of total emoluments payable on satisfactory completion of contract. Generous Education Allowances. Free passages. Furnished housing at moderate rental. Good leave. Free medical attention.

Further information and application forms from Secretary, The Council for Technical Education and Training for Overseas Countries, Eland House, Stag Place, London, S.W.1. Please quote TET/TKA/12(3). Closing date for receipt of applications November 8, 1965.

LIGHTING RESEARCH

A Lighting Engineer (Dip.M.I.E.S.) is required for an expanding daylight laboratory. This post provides scope for original work on prediction techniques and in subjective aspects of lighting. The Department also provides a daylight advisory service for architects: this is a fee-paying consultancy service so projects can be studied in depth. Equipment includes an artificial sky for model studies, and digital computer facilities are available.

No previous experience of daylighting is required, a degree would be an advantage but less important than an interest in interior lighting.

Please write for further information, quoting PAD/L, to L. P. Laidlaw, Pilkington Brothers Limited, Prescot Road, St. Helens, Lancashire.

The proposed
UNIVERSITY OF BATH
A TECHNOLOGICAL UNIVERSITY

(Bristol College of Science and Technology)

Applications are invited for the post of

SENIOR RESEARCH FELLOW
(Mathematics of Control)

to work on the mathematics of adaptive control systems. Candidates should have a good honours degree or Diploma in Technology and should have a strong interest in the application of mathematics to the analysis of real, physical systems. Preference will be given to candidates with experience in using the statistics of random processes in both linear and non-linear systems.

Salary range £1,400 to £2,165/£2,505 dependent on qualifications and experience. F.S.S.U.

Further details and application form from the Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7. Please quote reference CST 65/102.

UNIVERSITY OF NEWCASTLE UPON TYNE
THE MEDICAL SCHOOL

Applications are invited from Science Graduates holding M.Sc., Ph.D. or equivalent qualifications for the appointment of Senior Research Associate for research on isoenzymes in cancer, in the Department of Clinical Biochemistry. Some experience in protein chemistry is essential. Commencing salary will be £1,400 to £1,500 per annum according to the qualifications and experience of the successful candidate.

Applications, including the names and addresses of two referees, should be sent as soon as possible to Professor A. L. Latner, the Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne 1.

UNIVERSITY OF NEWCASTLE UPON TYNE

LECTURESHIPS IN ENGINEERING MATHEMATICS

The University invites applications for the post of Lecturer in Engineering Mathematics. This post, which has been newly-created, is for the current academic year and it is hoped that an appointment will be made as soon as possible. Two further Lectureships will be tenable from September 1, 1966. The commencing salary will be at an appropriate point on the Lecturers' scale £1,400 to £2,505. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, the University, Newcastle upon Tyne 2, with whom applications (three copies), together with the names and addresses of three persons to whom reference may be made, should be lodged within three weeks of the appearance of this advertisement.

The proposed

UNIVERSITY OF BATH
A TECHNOLOGICAL UNIVERSITY

(Bristol College of Science and Technology)

RESEARCH FELLOW

Applications are invited for the post of Research Fellow in the School of Physics, to work on new direct-gap semiconductor compounds for use as semiconductor lamps, lasers and Gunn devices. Applicants should possess a higher degree and experience in the field of semiconductor physics, technology or chemistry would be desirable. The appointment will be for three years in the first instance and will be tenable in Bristol.

Salary will be in the range: Fellow, £1,050 by £75 to £1,275; Senior Fellow no less than £1,400, according to age and qualifications. F.S.S.U.

Further particulars and application form from the Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7. Please quote reference CST 65/98.

UNIVERSITY OF WESTERN AUSTRALIA

APPOINTMENTS IN ANATOMY

Applications are invited for appointment to the following positions in the Department of Anatomy:

(1) LECTURESHIP; and
(2) TEMPORARY SENIOR LECTURESHIP for the year 1966.

The salary range for a Lectureship is £A2,400 by £A110 to £A3,170 per annum and for a Senior Lectureship, £A3,250 by £A110 to £A3,800 per annum. Superannuation similar to F.S.S.U. will apply to the Lectureship.

The Department provides courses for undergraduate and postgraduate students in the Faculties of Medicine, Science and Dental Science. It would be desirable to fill one of the vacancies by the appointment of a person with Histology or Cytology as a major in a B.Sc. degree in Anatomy (or with postgraduate experience in this field) and the other with a person primarily interested in developing the teaching and research in Physical Anthropology, Primatology or Human Biology.

Intending applicants are requested to obtain details of the procedure to be followed in applying for the post and a copy of the conditions of appointment before submitting their applications. This information is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 20, 1965.

PHYSICIST AND TECHNICIANS

Several vacancies exist in sections engaged on interesting applications of physical and physico-chemical methods in food research. Applicants of A-level, H.N.C. and graduate standards are required. Experience in either rheology or instrumentation and electronics will be an advantage though not essential.

Apply, in writing, to the Director of Research, The British Food Manufacturing Industries Research Association, Randals Road, Leatherhead, Surrey, giving qualifications and experience.

OPERATIONAL RESEARCH CONSULTING STAFF

Cooper Brothers & Co. require additional staff for their expanding management science division to assist in applying operational research to management problems in a variety of industries. The firm seeks

- ✱ Young graduate mathematicians, to engage initially in providing a specialist service to consultants on clients' assignments.
- ✱ Operational research scientists, who are graduates or otherwise professionally qualified, with at least three years' experience of O.R. in management problems, to undertake consultancy assignments.

Experience in using computers is desirable.

Vacancies exist at various levels and there are opportunities for working both at home and abroad. Training will be given as necessary. Starting salaries are high and there are excellent prospects for promotion. Applications should be sent in confidence to

Cooper Brothers & Co., MN.70, Abacus House, Gutter Lane, London, E.C.2.

WYETH

RESEARCH AND DEVELOPMENT

Senior Laboratory Technician

An experienced technician is required by the Pharmacology Department to assist in the evaluation of potential new drugs acting on the central nervous and cardiovascular systems.

Applicants must be familiar with modern recording equipment used in such investigations.

A competitive salary is offered with regular reviews, and the Company operates a non-contributory pension and life assurance scheme.

Please write with full details to Personnel Manager, John Wyeth and Brother Limited, Huntercombe Lane South, Taplow, Maidenhead, Berks.

FISONS PHARMACEUTICALS LIMITED

CHIEF PATHOLOGIST
FOR THERAPEUTIC RESEARCH

FISONS PHARMACEUTICALS LIMITED, wish to appoint a graduate in veterinary science or medicine as Chief Pathologist of its Research Laboratories. The successful candidate will be responsible to the Research Director for the Histology and Chemical Pathology Sections. The work will entail an evaluation of the toxicity of pharmaceutical compounds on a wide range of laboratory animals and will involve close co-operation with the Chief Toxicologist and senior members of staff. The post is suitable for a young graduate, with a few years' experience in a similar field, who has an experimental approach to Pathology and now merits greater responsibility.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications, which will be confidential, should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

LKB

A CONSULTANT
GAS CHROMATOGRAPHER

is required by LKB. This post is for a biochemist experienced in Gas Chromatography. Knowledge of mass spectroscopy would be a distinct advantage. After a period of training in Sweden the person would be required to visit various research centres throughout the United Kingdom and Western Europe. A good salary is being offered and full expenses will be paid, a Company car is provided.

Write in confidence to :

The Director,
LKB Instruments Ltd.,
LKB House,
137 Anerley Road,
London S.E.20.

BRADFORD INSTITUTE OF TECHNOLOGY

(Proposed University of Bradford)

RESEARCH ASSISTANTS IN ORGANIC CHEMISTRY

Applications are invited from graduates, Graduate Members of the Royal Institute of Chemistry or persons with equivalent qualifications for research appointments at the postgraduate and postdoctorate level in Organic Chemistry to work in one of the fields: aspects of nucleic acid chemistry, carbohydrates, plant hormones, reaction mechanisms associated with intermediates in biosynthesis. The work, which is suitable for submission for a higher degree, will be carried out in the Department of Chemical Technology (Head of Department, Professor R. L. Elliott), under the general supervision of Dr. G. Shaw. Salary within the scale £750 to £1,350 will be according to experience and qualifications.

Application forms may be obtained from the Registrar, Ref. 125Y/X, Bradford Institute of Technology, Bradford, 7.

UNIVERSITY COLLEGE, DUBLIN

FACULTY OF GENERAL AGRICULTURE

COLLEGE LECTURESHIP IN GENETICS IN THE AGRICULTURAL BIOLOGY DEPARTMENT

Applications are invited for the above post. Candidates must have pursued postgraduate work and have teaching experience in Genetics. The salary scale is £1,500 by £50 to £2,100. Non-contributory pension and family allowances are additional to salary.

Applications should state age, qualifications and experience, together with the names of two referees, and should reach the undersigned not later than October 22, 1965.

J. P. MacHALE,
Secretary and Bursar.

UNIVERSITY OF MANCHESTER

FACULTY OF TECHNOLOGY

Applications are invited from graduates with an honours degree in Chemistry, Textile Chemisty or Chemical Engineering for awards tenable in the Department of Polymer and Fibre Science, concerned with the study of mass transfer relationships when dyes are taken up from solution by polymers and fibres as follows: Research Assistant, salary in the range £900 to £1,100 per annum with F.S.S.U. Research Studentship, stipend in the range £500 to £700 per annum.

Applications should be addressed as soon as possible to the Registrar, The Manchester College of Science and Technology, Manchester, 1, from whom application forms can be obtained.

BIOLOGIST

TANZANIA

A biologist is required to investigate the ecology of game animals in the woodland areas. Candidates (between 24 to 35 years) should hold an honours degree in Zoology with postgraduate experience in animal ecology. Salary, £1,281 to £2,757 per annum, plus 25 per cent terminal gratuity. Passages provided. Education allowances. Furnished accommodation. Generous home leave. Appointment on contract for one or two tours of 21 to 27 months each tour.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting Ref.: RC 275/173/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

THE AGRICULTURAL RESEARCH COUNCIL has a vacancy for a Microbiologist/Bacteriologist at their Pest Infestation Laboratory to work on the diseases of insects that infest stored food materials. The appointment will be in the Experimental Officer class and the point of entry will be according to age and experience. Minimum qualifications: G.C.E. in five subjects, including two scientific subjects at Advanced level or equivalent; or, at age 22, pass degree or equivalent. Salary for A.E.O. according to age in scale £549 to £776 (at age 22) to £1,201; for E.O. £1,319 to £1,675. Contributory superannuation scheme.—Apply to: Director, Pest Infestation Laboratory, London Road, Slough, Bucks.

GROUP LABORATORY, WATFORD. Senior I required for Chemical Pathology Department. A busy routine laboratory offering an opportunity for gaining experience in modern techniques and Steroid Analysis. Salary scale according to Whitley Council Regulations.—Applications, giving full particulars of age, qualifications, experience and names of two referees, to Consultant Chemical Pathologist, Group Laboratory, 6 Rickmansworth Road, Watford, Herts.

CHEMICAL ENGINEER REQUIRED TO take part in a programme of research and development. The work may be either on pilot plant or full-scale operations. The man appointed will be expected to undertake projects with minimum supervision. Salary according to age and experience. Non-contributory pension scheme.—Apply to the Manager, The Distillers Co. Ltd., Glenochil Research Station, Menstrie, Clackmannanshire, Scotland.

UNIVERSITY COLLEGE, DUBLIN

DEPARTMENT OF AGRICULTURAL CHEMISTRY

ASSISTANT LECTURER OR COLLEGE LECTURER

Applications are invited for the post of Assistant Lecturer or College Lecturer in Agricultural Chemistry. Applicants should have an honours degree in Agricultural Science, Chemistry or Biochemistry with suitable postgraduate experience. The salary scales are: Assistant Lecturer, £1,200 by £50 to £1,450; College Lecturer, £1,500 by £50 to £2,100. A non-contributory pension scheme and family allowances are additional to salary.

Applications, with the names and addresses of two referees, should be submitted before October 22, 1965.

J. P. MacHALE,
Secretary and Bursar.

PLANT PHYSIOLOGIST

EAST AFRICAN COMMON SERVICES ORGANISATION

Required to carry out research on agreed problems in the physiology of root development and growth in some East African economic plants, with particular reference to the effects of water stress.

Candidates must hold a good second-class honours degree in botany, with particular emphasis on physiology ecology. Postgraduate experience in plant physiology research, with particular reference to water shortage on plant growths is desirable.

Salary scale £1,221 to £2,097 a year plus 25 per cent terminal gratuity. An increase of 12½ per cent of existing salaries has been approved. Quarters available. Passages provided. Two-year contract. Education allowances. Generous leave.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience quoting RC 213/214/010 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

GALWAY COUNTY COUNCIL

VACANCY FOR SENIOR LABORATORY TECHNICIAN FOR NATIONAL SALMONELLA AND SHIGELLA CENTRE AT GALWAY REGIONAL HOSPITAL

Application forms and full particulars in connection with above post may be obtained from the Secretary, Galway County Council, County Buildings, Galway, to whom completed applications should be returned so as to reach him on or before November 5, 1965.

Qualifications: Each candidate must: (a) be a member of the Institute of Medical Laboratory Technology or be deemed by virtue of his training and experience to have reached an equivalent technical standard; (b) have had adequate practical training and experience in a recognized medical laboratory; (c) be competent to keep records appropriate to his service in the laboratory. Age: Each candidate must have attained the age of 20 years on or before November 1, 1965.

QUEEN MARY COLLEGE

(UNIVERSITY OF LONDON)

Applications are invited for the post of Lecturer in the Department of Nuclear Engineering. Either a Theoretical Reactor Physicist with experience in modern computational techniques or a candidate well qualified in the fields of fluid dynamics and heat transfer is required. Applicants with the latter qualifications need not necessarily have a nuclear engineering background. Departmental activities include research in the above fields as well as graduate and undergraduate teaching. Salary scale: £1,400 by £85 to £2,165 (efficiency bar) by £85 to £2,505, plus London Allowance of £60 and F.S.S.U. participation. Initial salary according to qualifications and experience.

Application forms and further details obtainable from the Registrar, Queen Mary College, Mile End Road, London, E.1, to be returned by November 5.

UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND

Applications are invited for the following appointments in the Department of Biological Sciences:

CHAIR OF PLANT SCIENCE
LECTURESHIPS IN BIOLOGICAL SCIENCE

Inquiries welcomed from persons interested in secondment for a limited period. Applications would be particularly welcome from persons with special interests in one or more of the following fields: parasitology, vertebrate zoology, entomology, medical biology, plant ecology, microbiology, plant physiology, and plant morphology. The Chair of Plant Science will be a second chair in the newly formed Department of Biological Sciences. Salary scales: Professor, £3,600 by £150 to £4,200 per annum; Senior Lecturer, £2,520 by £120 to £3,480 per annum; Lecturer Grade I, £2,256 by £96 to £2,640 per annum; Grade II, £1,740 by £84 to £2,160 per annum; Assistant Lecturer, £1,400 by £75 to £1,625 per annum. Family passages and allowance for transport of effects on appointment. Installation grant of £100 for persons recruited from outside Africa. Travel assistance for appointee for triennial (Professors, biennial) visits overseas and for appointee and family for six-yearly sabbatical leave and on retirement or resignation after five years' service. Superannuation and medical aid schemes. Unfurnished College accommodation at rent of 15 per cent of salary if desired.

Detailed applications (six copies), naming three referees, by November 4, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

ENTOMOLOGIST

TANZANIA

Applications are invited from suitably qualified persons able to carry out major surveys of tsetse infested country, to plan and estimate the cost of control measures and to finally evaluate the results. A certain amount of laboratory work will be involved but duties lie mainly in the field. The selected officers must be prepared to travel and camp in remote areas. Candidates male, who should normally be nationals of the United Kingdom or the Republic of Ireland, should possess an honours degree in Entomology, Biology or Zoology (with an emphasis on Entomology). Salary £1,281 to £2,757 per annum (including Overseas Allowance), depending upon experience, plus 25 per cent terminal gratuity. Education allowances. Free passages. Accommodation available. On contract for one or two tours of 21 to 27 months.

Please apply for further details, giving full name, qualifications, experience and quoting Ref. RC 217/173/07 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF MANCHESTER

The Department of Metallurgy requires an Experimental Officer to undertake work in electronics. Much of the research work of the Department utilizes electronic equipment. Whilst some of this can be purchased, much of the equipment must be designed and constructed for a particular function. This requires a man who is knowledgeable about electronic techniques and devices and is able to apply this knowledge to the designing of circuits which must then be constructed, tested, and put into operation. The Experimental Officer will also be expected to maintain electronic equipment within the Department and to advise on all future purchases of such apparatus. Applicants should preferably possess H.N.C., Dip.Tech., or some professional qualification, including electronics. Experience is essential. The initial salary will be within the range £870 to £1,450 per annum, according to qualifications and experience.

Applications should be submitted by October 23, 1965, to the Registrar, the University, Oxford Road, Manchester 13, from whom further particulars and forms of application may be obtained on quoting reference 175/65/Na.

UNIVERSITY OF ILLINOIS

COLLEGE OF PHARMACY
833 SO. WOOD STREET,
CHICAGO, ILLINOIS 60612

Applications are invited for the post of Instructor or Laboratory Assistant in Pharmacognosy for the Academic Year 1966-67. Applicants should have M.S. or be approaching Ph.D. Salary commensurate with training and experience. Give complete Educational History and experience.

Applications should reach Dr. Ralph F. Voigt, Head of Department of Pharmacognosy and Pharmacology by November 15, 1965.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

(Proposed Welsh University of Technology)
CATHAYS PARK, CARDIFF
DEPARTMENT OF APPLIED PHYSICS
ELECTRON-MICROSCOPE TECHNICIAN

to assist with the operation and maintenance (day-to-day only) of the Department's JEM-7 electron-microscope, and preparation of specimens. Previous experience in electron-microscopy is desirable but not essential (training can be provided), but applicants must hold at least, a H.N.C. or pass degree (or equivalent) in Applied Physics or Physics. Salary will be on the Scale for Senior Technicians (Grade B) £920 to £1,040 plus a supplementary award of £75 to holders of a H.N.C. or degree.

Application forms and further particulars may be obtained from the Registrar, to whom completed forms should be returned by October 21, 1965.

ASSISTANT CONSERVATOR OF FORESTS

KENYA

Required for work in connection with the administration and technical direction of a forest division. Candidates, under 40 years should hold a degree in forestry. Salary, £1,374 to £2,757 plus 25 per cent terminal gratuity. Education allowances. Passages provided. If Government quarters unavailable, furnished accommodation usually obtainable at low rental. Generous home leave. Two-year contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details giving full name, and brief particulars of qualifications and experience, quoting Reference RC 324/95/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

KENYA POLYTECHNIC

Applications are invited for the post of Assistant Lecturer (Grade I) in Physics to teach Physics and Pure or Applied Mathematics to G.C.E. Advanced level. Candidates should be nationals of U.K. or Republic of Ireland and have honours degree in Physics and Diploma in Education with five years teaching and/or industrial experience. Appointment on contract for two years in the first instance. Salary £1,819 to £2,278 per annum according to qualifications and experience. Resettlement grant of 25 per cent of total emoluments payable on satisfactory completion of contract. Generous education allowances. Furnished housing at moderate rental. Free passages. Good leave. Full medical treatment.

Further information and application forms from Secretary, The Council for Technical Education and Training for Overseas Countries, Eland House, Stag Place, London, S.W.1. Please quote TET/KP/12 (5). Closing date for applications November 1, 1965.

SCHOOL OF PHARMACY

UNIVERSITY OF LONDON
LECTURESHIP IN PHARMACOLOGY

Applications are invited for appointment as a Lecturer in the Department of Pharmacology. The successful applicant will ideally have sufficient experience to justify his appointment at a salary in the upper half of the Lecturer's scale. He will be expected to carry out teaching and personal research in a field acceptable to the Head of the Department. It is desirable that he should be able to take up the appointment early in 1966. Normal University Terms of Service will apply to this appointment including a London Allowance of £60 per annum.

Applications should be returned by October 30, 1965, to the Clerk to the Council, School of Pharmacy, University of London, 29/39 Brunswick Square, W.C.1, from whom further information may be obtained.

FISHERIES OFFICER, BRUNEI. APPLICATIONS are invited from suitably qualified persons for this post to advise the Development and Marine Department on all aspects of the development of the Brunei Fishing Industry. Candidates should hold a degree in biology with relevant postgraduate experience. Salary £2,982 per annum plus 12½ per cent terminal gratuity. Passages provided for officer and family. Education and Outfit Allowances. Generous leave. Two- to three-year contract. Candidates should apply for further details, giving full name and brief particulars of qualifications and experience, quoting Ref.: RC 289/28/01 to: Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1.

CHARING CROSS HOSPITAL

Physics Laboratory Technician required for Group Isotope Laboratory. Entry either as Technician-in-Training, salary £276 at age 16 to £552 at age 25 or over, plus London Weighting £25; or Technician, minimum age 21 years, salary £668 to £883 plus £25 London Weighting, with minimum qualifications of Ordinary National Certificate in Applied Physics or equivalent. Applicants with higher qualifications or more experience may enter above the minimum of the Technician's scale.

Applications with the names of two referees to reach House Governor (A), Charing Cross Hospital, W.C.2, not later than October 23, 1965.

SCOTTISH MARINE BIOLOGICAL ASSOCIATION

The Council of the Association will proceed, if possible in the spring or certainly in the summer of 1966, to appoint a Director of the Marine Biological Laboratory at Millport on the resignation of Dr. C. H. Mortimer, F.R.S.

Those who wish their names to be considered are requested as early as possible but not later than November 30 to inform the President, Dr. C. M. Yonge, C.B.E., F.R.S., 13 Cumin Place, Edinburgh 9. The terms of appointment will be the subject of discussion between the Council and the selected candidate.

RESEARCH CHEMISTS

Able young Post-doctoral Organic Chemists, required to work in a modern well equipped laboratory on the isolation and synthesis of Pharmacologically active compounds, experience in natural product work is desirable.

Apply to the Managing Director, Biorex Laboratories Ltd., Research Division, Apsley House, 198 City Road, London, E.C.1.

BRUNEL COLLEGE

DEPARTMENT OF BIOLOGY

Applications are invited for the following posts:

ASSISTANT LECTURER IN BOTANY
ASSISTANT LECTURER IN ZOOLOGY

Candidates should hold a good honours degree or equivalent, and preferably have had research or industrial experience. Salary within the range, £1,110 to £1,335 per annum.

Further particulars from the Academic Registrar, Brunel College, Woodlands Avenue, London, W.3. Closing date: October 22, 1965.

CITY OF LIVERPOOL MUSEUMS

Applications are invited for appointment as Keeper of Botany. Salary £1,610 to £1,940 (Grade B). Qualifications must include a good honours degree in botany and/or the Museums Association Diploma; an interest in Economic Botany, and Museum experience of fieldwork. A working knowledge of at least one modern foreign language is also desirable.

Further particulars from the Director, City of Liverpool Museums, William Brown Street, Liverpool, 3. Closing date October 23, 1965.

THOMAS ALKER,
Town Clerk.

UNIVERSITY OF LIVERPOOL

Applications are invited for the post of Electronics Technician in the Department of Inorganic, Physical and Industrial Chemistry. The work will be associated with research studies of dielectric, ultrasonic and viscoelastic relaxation phenomena, and will be concerned primarily with the generation, gating and detection of a.c. signals. Initial salary up to £1,090.

Application forms may be obtained from the Registrar, The University, Liverpool 3. Please quote Ref. 299/N.

SENIOR SCIENTIFIC OFFICER OR SCIENTIFIC Officer required for breeding work and study of breeding methods with vegetables. Applicants should have a Ph.D., or good honours degree with equivalent research experience in genetics or plant breeding. Salary £1,685 to £2,082 (S.S.O.) or £895 to £1,521 (S.O.), scale and starting point depending on qualifications and experience. F.S.S.U.—Applications, with names and addresses of two referees, to be sent to the Secretary, National Vegetable Research Station, Wellesbourne, Warwick, by October 29, 1965.

UNIVERSITY COLLEGE LONDON: PROgrammer required for work on investigating Modern English Grammar (Survey of English Usage).—Applications, giving curriculum vitae, qualifications, and names of two referees, to the Secretary, University College London, Gower Street, W.C.1, by November 1, 1965.

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY

Applications are invited for the post of Research Assistant in Potato Branch for work on Herbicides in relation to potato varieties. This is an investigational project financed by the Potato Marketing Board and will be tenable in the first instance for a period of three years subject to annual review. Preference will be given to candidates possessing a degree in Botany, Agriculture, Agricultural Botany or other equivalent qualifications. Commencing salary £699 at age 21, £962 age 26 or over. Maximum of scale £1,610. Superannuation under F.S.S.U.

Application form and particulars from the Secretary, N.I.A.B., Huntingdon Road, Cambridge.

ROYAL HOLLOWAY COLLEGE

UNIVERSITY OF LONDON

DEPARTMENT OF PHYSICS

Applications are invited for a post of Lecturer or Assistant Lecturer in Theoretical Physics. The Physics Department is enlarging as part of the expansion of the College, and intends to build up a strong theoretical group in laser physics, including non-linear effects. Further details may be obtained from the Secretary, Royal Holloway College, Englefield Green, Surrey.

Four copies of applications, together with the names and addresses of two referees, should reach him as soon as possible.

LABORATORY TECHNICAL OFFICER

Applications are invited for the above post from graduates in chemistry. The successful candidate will be responsible to the Chief Technical Adviser for the supervision of the Technical Department laboratories, for the analysis of the Company's products, and the running of a soil testing service for the Company's farmer customers. Experience in the use of automated methods of analysis would be an advantage. The appointee will be based at the Company's Headquarters at Hartham, Corsham, Wiltshire.

Forms of application, which should be returned within two weeks of the appearance of this advertisement, can be obtained from:

The Chief Technical Adviser,
Soil Fertility Limited,
Hartham, Corsham,
Wiltshire.

KENT EDUCATION COMMITTEE

MEDWAY COLLEGE OF TECHNOLOGY

DEPARTMENT OF SCIENCE

Lecturer in Physical Chemistry required to teach to B.Sc. and Grad.R.I.C. Part II level. Applicants should be well qualified academically and have postgraduate experience in industry and/or teaching. The successful candidate will be given the opportunity to undertake research, for which good facilities are available. Salary: £1,670 to £1,895 (at present under review).

Application form and further particulars from the Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

APPLICATIONS ARE INVITED FOR THE position of Head of Department and Staff positions in the Department of Chemistry and Chemical Technology in the West Kootenay Regional College, Castlegar, B.C., Canada. The College will open in September, 1966, to some 400 students and will stress excellence in teaching and will encourage an experimental approach to the curriculum.

The positions will involve lecturing and conducting laboratory courses in inorganic, organic, general and analytical chemistry, and in engineering unit operations. Two programmes are envisaged: a two-year diploma course in chemical technology at the sub-professional level and chemistry courses equivalent to the first two years of a university programme. Applicants should possess a Ph.D. or Master's Degree with appropriate experience. Appointment will be made immediately and salaries and duties will begin June-July, 1966.

Starting salaries of up to $10,000 per annum are offered depending on qualifications and experience. Excellent pension plan and other benefits. Travel and removal grants available.

Please send curriculum vitae and the names of three referees to: The Principal, West Kootenay Regional College, 1385 Cedar Avenue, Trail, British Columbia, Canada.

CHEMICAL OR BIOCHEMICAL TECHNIcian required to assist in research on muscle disease.—Apply in writing, stating age, qualifications and experience to: The Secretary, Institute of Neurology, The National Hospital, Queen Square, W.C.1.

A SERVICE ENGINEER AND TECHNICAL REPRESENTATIVE

Experienced in electro-mechanical laboratory instruments is being sought by LKB. The post is available in November and the person will be required to work in London and the Home Counties. A good salary and Company car is provided, all expenses paid in full.

Write in confidence to The Sales Director, LKB Instruments Ltd., LKB House, 137 Anerley Road, London, S.E.20.

INSTITUTE OF CANCER RESEARCH: Royal Cancer Hospital. Junior Technical Officer required in Biochemistry Department, Chester Beatty Research Institute, Fulham Road, S.W.3. Candidates preferred with two G.C.E. "A" level passes in chemistry and one biological subject. Starting salary in range £575 to £750 according to age and experience, in scales continuing to £1,200 per annum.—Apply with names of two referees, to the Secretary, 34 Sumner Place, S.W.7, quoting ref. 301/B/219.

FELLOWSHIPS AND STUDENTSHIPS

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF PROTEIN CHEMISTRY

POSTDOCTORAL FELLOWSHIP

Applications are invited from Physicists or Chemists for appointment to a Postdoctoral Fellowship, tenable with the Organization's Division of Protein Chemistry located at Parkville, Melbourne, Victoria.

The Division has a research staff of some 30 organic and physical chemists, biochemists and biophysicists engaged in studies of the structure and chemistry of proteins. The equipment available for these studies includes microfocus x-ray generators, low-angle focusing cameras and a high-resolution prism grating infra-red spectrometer equipped with microscope and polariser. Facilities are also available for electron microscopy, n.m.r., e.s.r. and a wide range of physico-chemical and analytical techniques.

The Fellow will be invited to study the relationship between the molecular structure and mechanical properties of keratin, collagen, muscle proteins and synthetic analogues such as sequential polypeptides using x-ray diffraction at both high- and low-angles in conjunction with parallel studies using infra-red spectroscopy and electron microscopy.

Applicants should have a Ph.D. degree in Physics or Chemistry, or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability. Some experience with x-ray diffraction techniques or with mechanical studies of polymers would be an advantage.

SALARY: Dependent upon qualifications and experience within the ranges of: £A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

The Fellowship is offered for a period of three years. Consideration will be given to the continuation of F.S.S.U. arrangements.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of the appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No: 462/210) should be addressed by November 13, 1965.

UNIVERSITY OF QUEENSLAND

POSTGRADUATE RESEARCH FELLOWSHIP —PARASITOLOGY

Applications are invited for the tenure of a postgraduate research fellowship within the Department of Parasitology. The appointee will be required to work towards a Ph.D. on a subject relating to infection with filarioid nematodes of man or animals in Queensland and New Guinea. The salary will be within the range £A750 to £A1,500 according to experience and qualifications. Written applications should be forwarded to the undersigned.

Applications close on November 30, 1965. Applicants from the United Kingdom should apply to the Queensland Government Offices, 409-410 Strand, London, W.C.2, for further particulars about this position.

C. J. CONNELL,
Registrar.

University of Queensland,
St. Lucia, Brisbane,
Queensland, Australia.

POSTDOCTORAL FELLOWSHIP ($6,000 tax-free stipend) in protein chemistry available early 1966 for applicants with previous experience in this area. Study will involve physico-chemical, chemical and enzymatic investigations. —Apply with curriculum vitae and two letters of reference to Dr. C. M. Kay, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

UNIVERSITY OF KEELE

DEPARTMENT OF COMMUNICATION

A five-year research programme on perceptual problems of the blind and deaf will have a vacancy for a Research Fellow from January 1, 1966. Salary, according to qualifications, on the Assistant Lecturer/Lecturer scale with F.S.S.U. A multidisciplinary approach is projected, combining the techniques of experimental psychology, electro-physiology and electronics.

Candidates with experience in one or more of these fields are invited to apply to the Registrar, the University, Keele, Staffs., from whom application forms can be obtained for return by November 15, 1965.

UNIVERSITY OF SOUTHAMPTON

POSTDOCTORAL FELLOWSHIP

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Applications should be sent to the Deputy Secretary, The University, Southampton by October 18, 1965.

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Dr. H. Rosenberg—Phosphate and phosphonate metabolism, and transport in micro-organisms.

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Further particulars and information as to the method of application should be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 15, 1965.

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Applications for grants from the second allotment of the Government Grant for Scientific Publications for the year 1965 should be made as soon as possible. No application can be considered which is received later than November 15, 1965. The grant is provided for the assistance of scientific publications in the United Kingdom, particularly but not exclusively those of scientific societies and institutions of a national character. A small part of the grant is available for the assistance of the libraries of scientific societies. Applications are receivable only from societies and institutions in the United Kingdom and individuals of British nationality.

Forms of application may be obtained from the Executive Secretary, The Royal Society, Burlington House, London, W.1.

COURTAULDS' EDUCATIONAL TRUST FUND

The Committee administering the Trust Fund proposes in April 1966 to make grants to certain educational establishments for the purchase of capital equipment.

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The Secretary to the Committee,
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London, W.1.

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COMMONWEALTH SCHOLARSHIP AND FELLOWSHIP PLAN

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(2) Canada at Canadian universities from September, 1966.

(3) East Africa at the University of East Africa from October, 1966.

(4) Ghana at Ghanaian universities from October, 1966.

(5) Hong Kong at the University of Hong Kong from September, 1966.

(6) Jamaica at the University of the West Indies from October, 1966.

(7) Nigeria at Nigerian universities from October, 1966.

Commonwealth Scholarships are in general tenable for a programme of study or research normally extending over a period of two academic years and leading to a higher degree or similar qualification. The awards are normally open to men and women students under the age of 35 whose permanent homes are in Britain and who are citizens of the United Kingdom or some other Commonwealth country. Emoluments will cover fares, fees, and maintenance allowances of:

(1) £A900 per annum in Australia; (2) $165 per month in Canada; (3) £600 per annum in East Africa; (4) £G720 in Ghana; (5) £56 15s. per month in Hong Kong; (6) £50 per month in Jamaica; (7) £700 per annum in Nigeria.

A marriage allowance may be paid in certain circumstances to Scholars studying in Australia, Canada, East Africa, Hong Kong, and Jamaica. Certain other allowances are also payable.

Further particulars and application forms may be obtained from the Secretary-General, The Association of Commonwealth Universities, Marlborough House, Pall Mall, London, S.W.1, with whom applications must be lodged not later than November 3, 1965.

TRAVELLING AND RESEARCH GRANTS. Fellowships for Women Graduates 1966-67, value $2,000 to $2,500, together with some smaller awards. Closing date October 22, 1965. Details from the Academic Secretary, British Federation of University Women, Ltd., Crosby Hall, Cheyne Walk, London, S.W.3.

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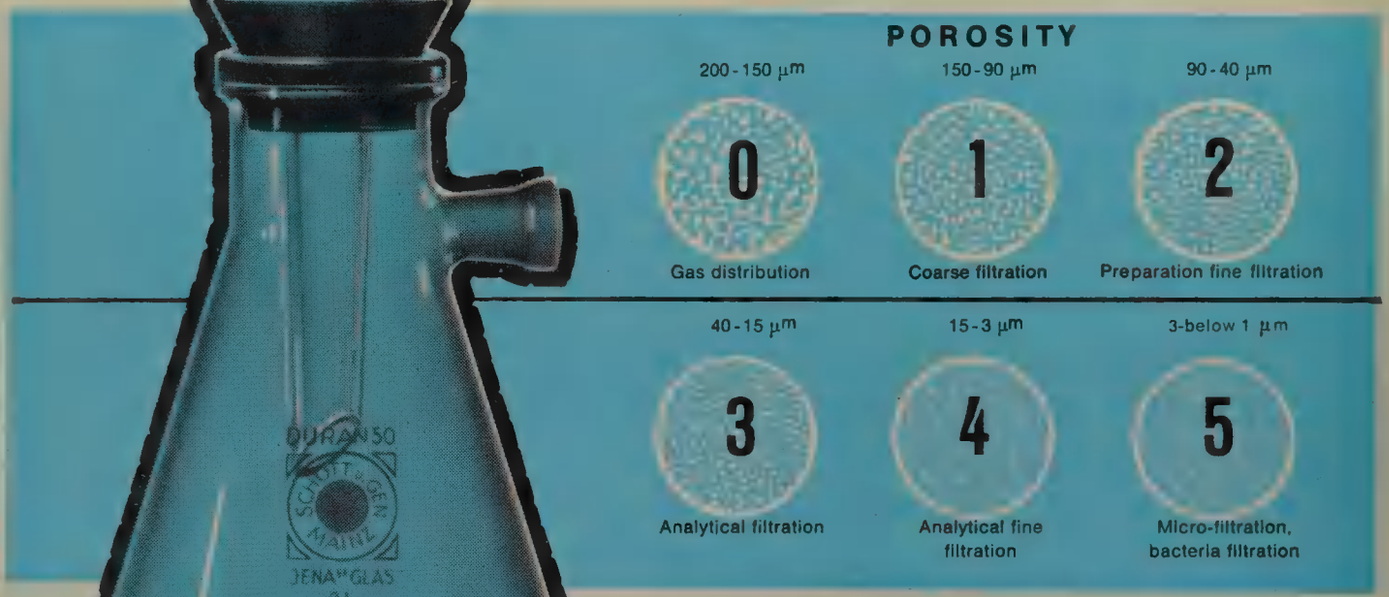

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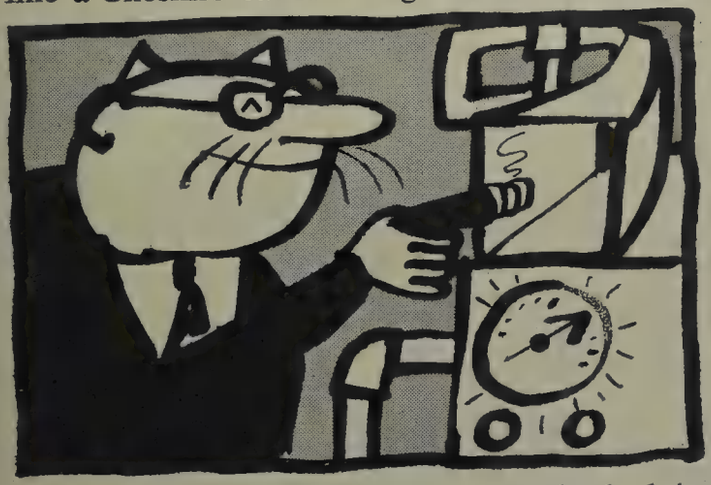
The story is told that when the first crude version of the Dicke Microwave Radiometer — the heart of most present day radio telescopes — was being tested, one of the division heads expressed doubt that a piece of electronic hardware could be cajoled into responding to ordinary thermal radiation at microwave frequencies. The noise level in the primitive receivers of that time was at least 10 db above ambient thermal radiation levels, and it had been claimed that the Dicke radiometer had a sensitivity of 1/2°C. To make his point, this well-known physicist took his lighted cigar and held it up to the input wave guide of the receiver. When to his surprise the meter banged off scale, he smiled like a Cheshire cat in a cage full of ducklings.



The heart of the radiometer was the lock-in amplifier, and the technique of fishing a small signal out of a thick porridge of noise became known thereafter as the *"lock-in amplifier technique."* After the initial success with a lock-in amplifier the technique was used in a variety of places, including nuclear magnetic resonance measurements and microwave spectroscopy experiments. The practitioners of this black art have now increased to a reasonably large number, but a far larger number of scientists in all fields are still up to their necks trying to find a weak signal in a gooey background of noise.

Possible applications for the lock-in amplifier technique are almost endless, but in some scientific fields the amplifier is not even known, much less appreciated. The biological sciences offer many interesting challenges. Has anyone ever shone a light in a cat's eyes to see if it elicits a motor response? If the light were chopped at 5 cycles/sec. and a lock-in amplifier were connected to an appropriate strain gauge, this response could be studied, not only under conditions of very weak signal, but also as a function of chopping frequency.

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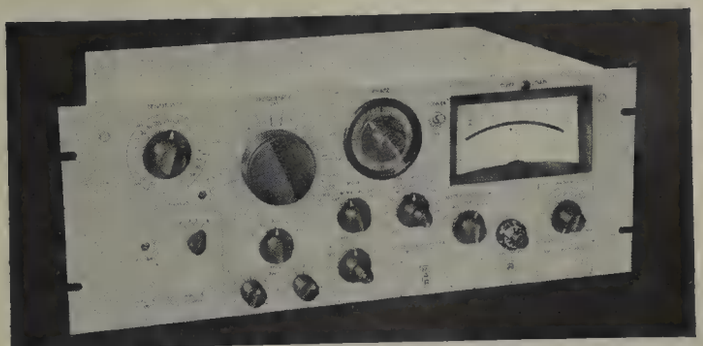

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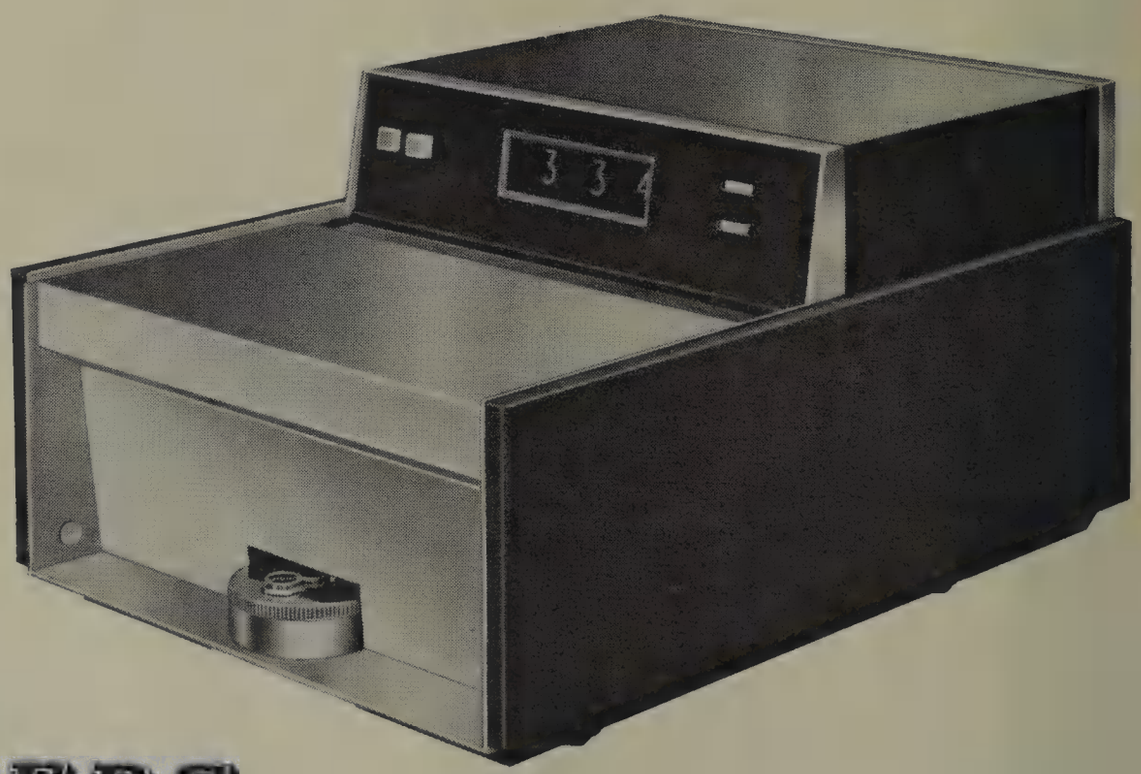

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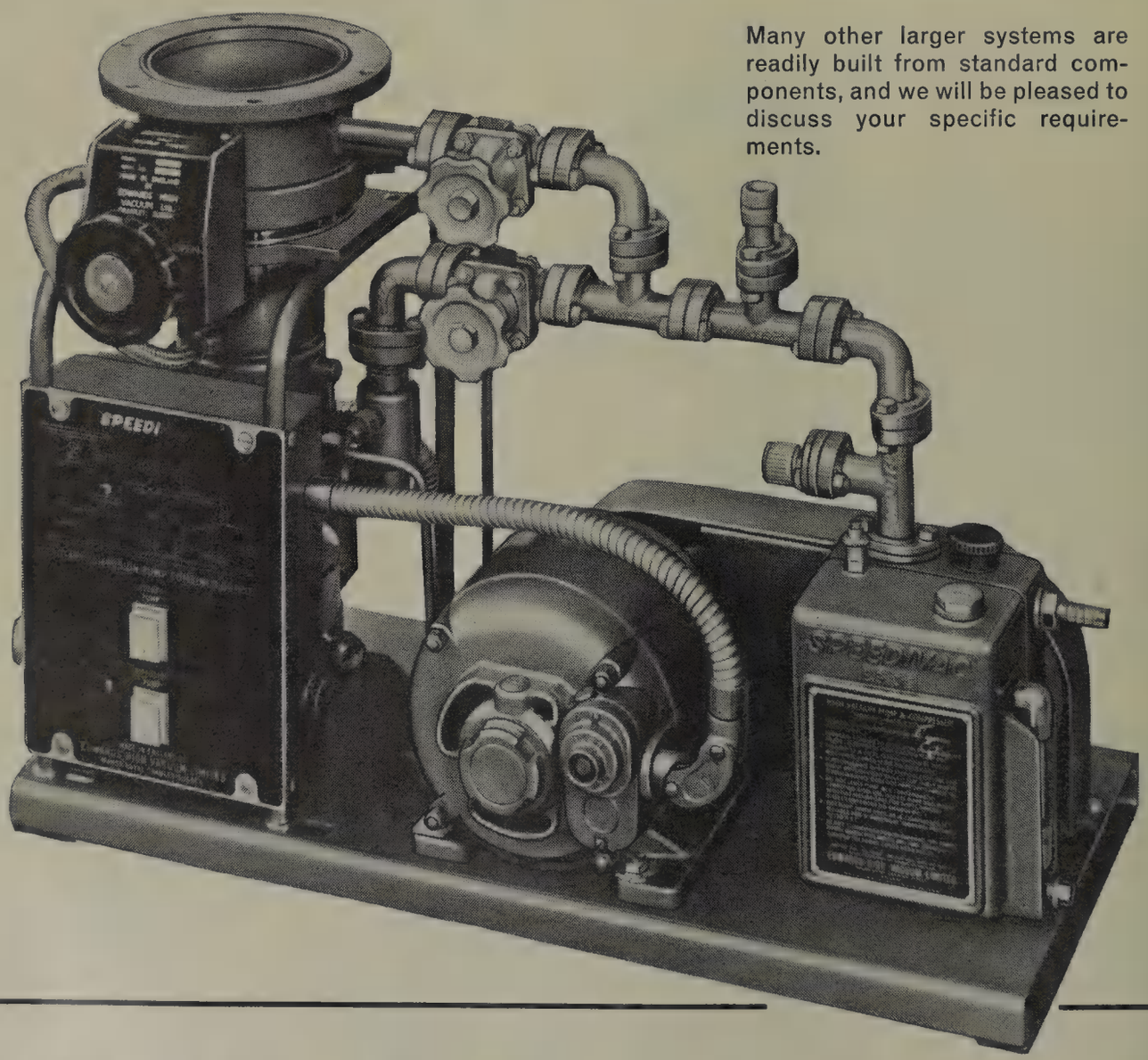

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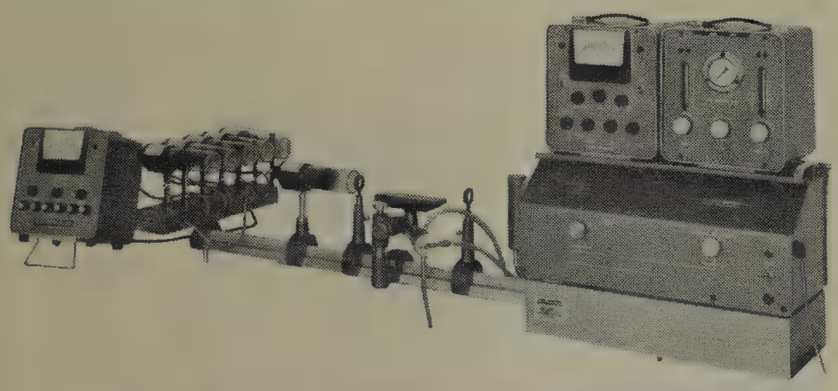

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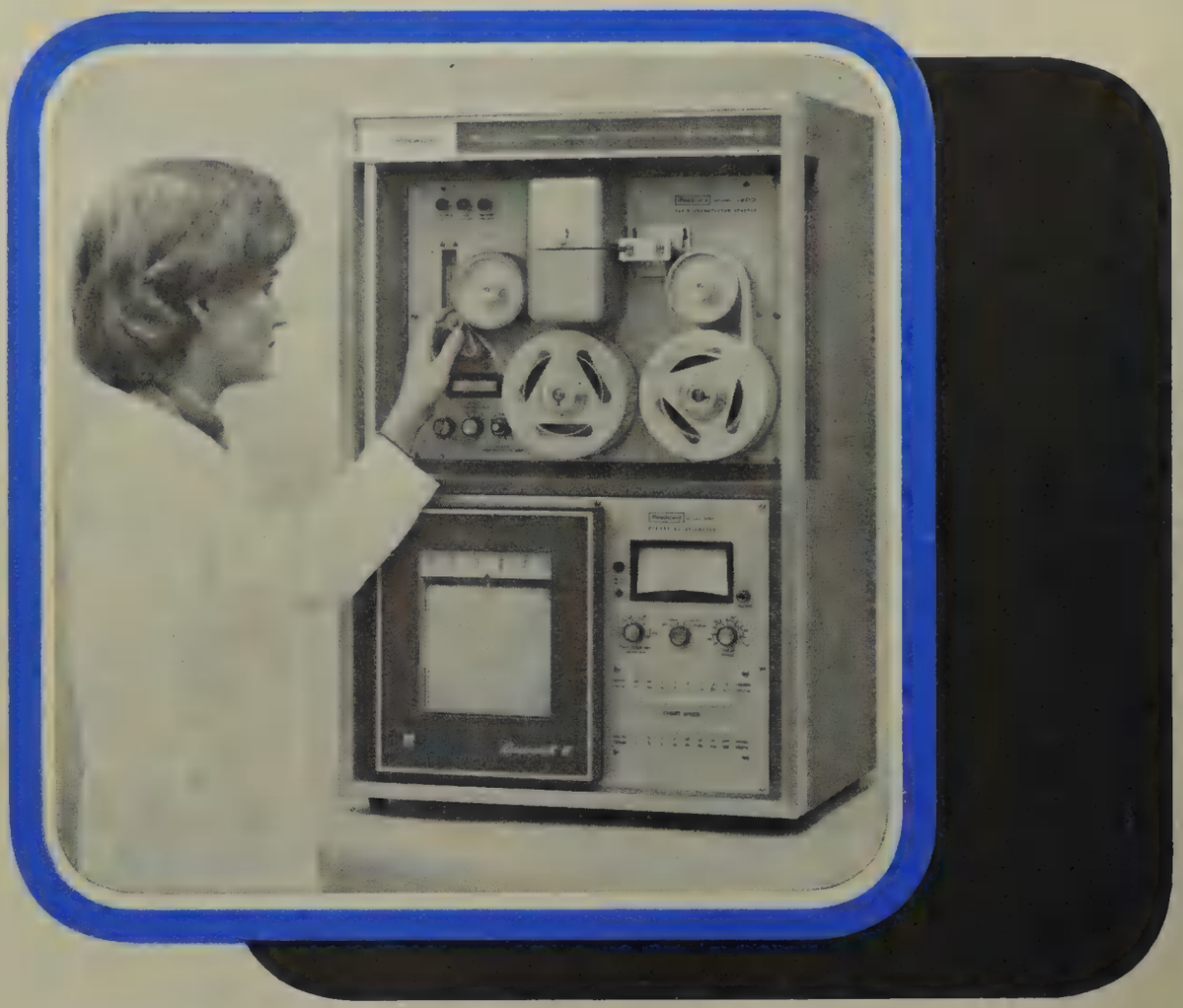

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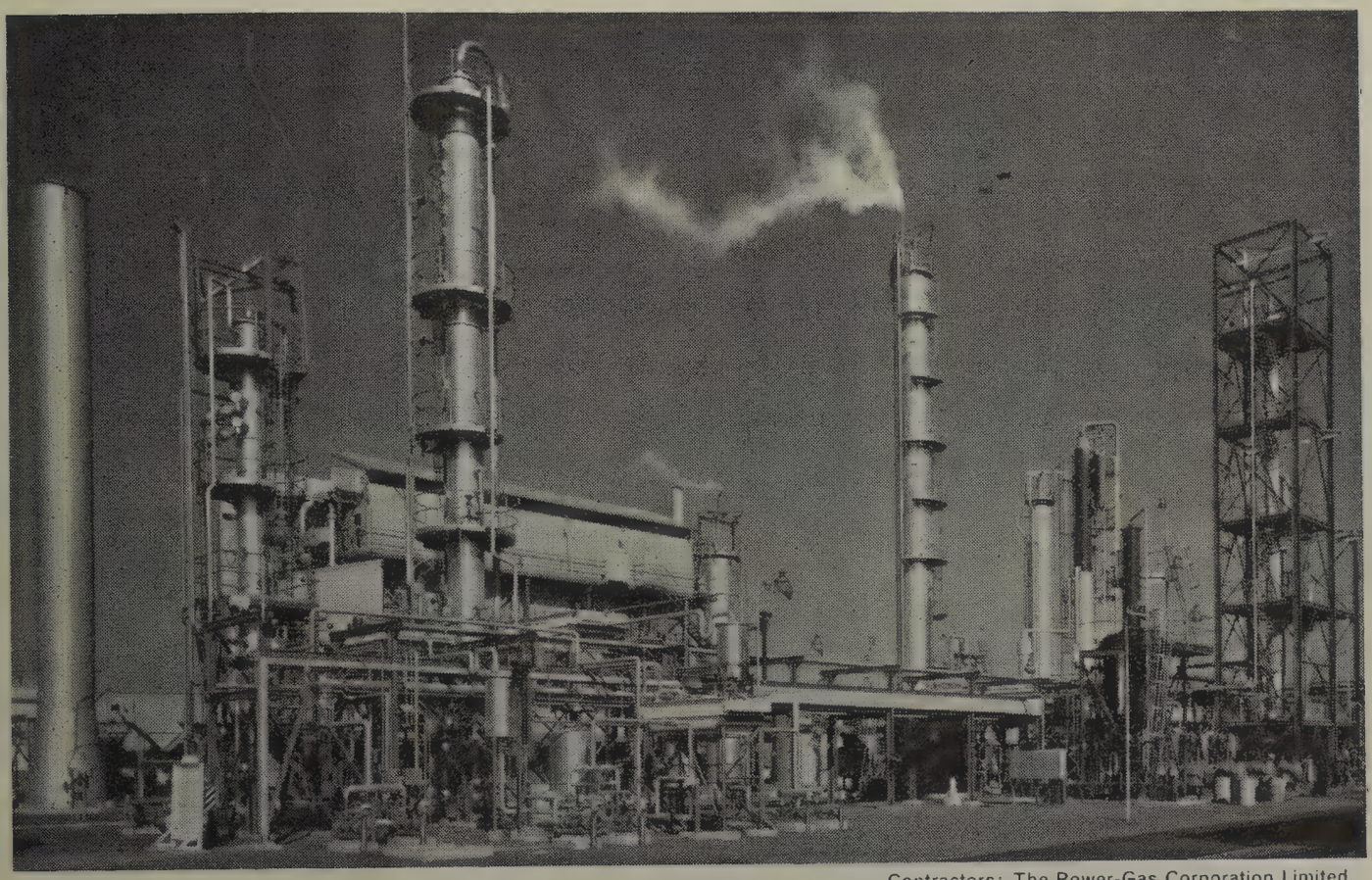

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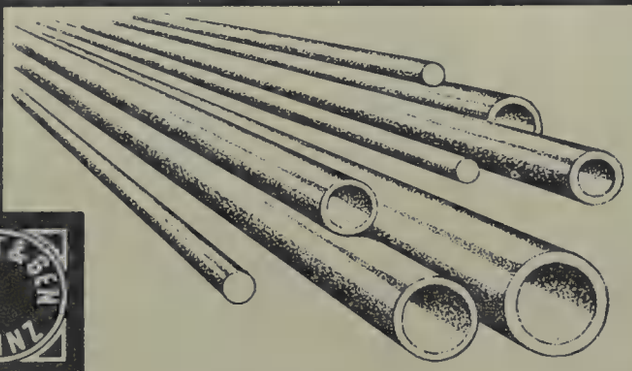

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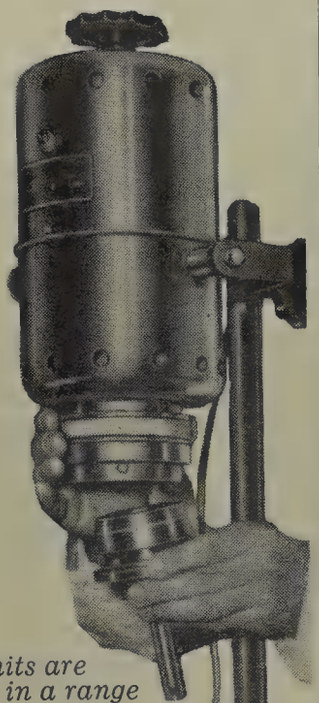

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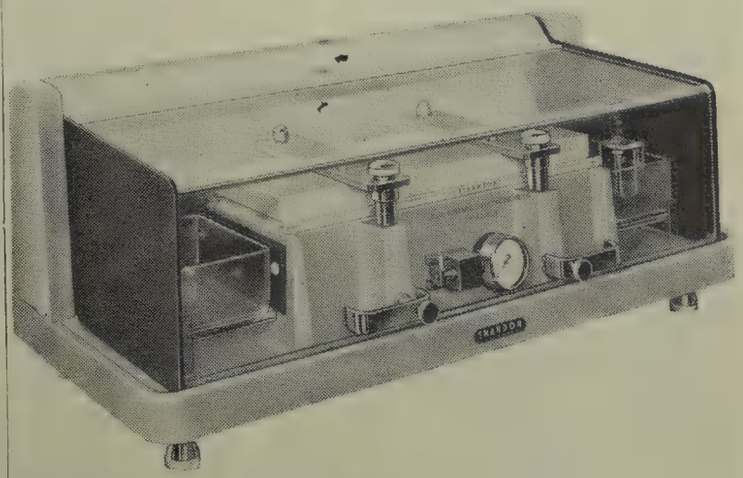

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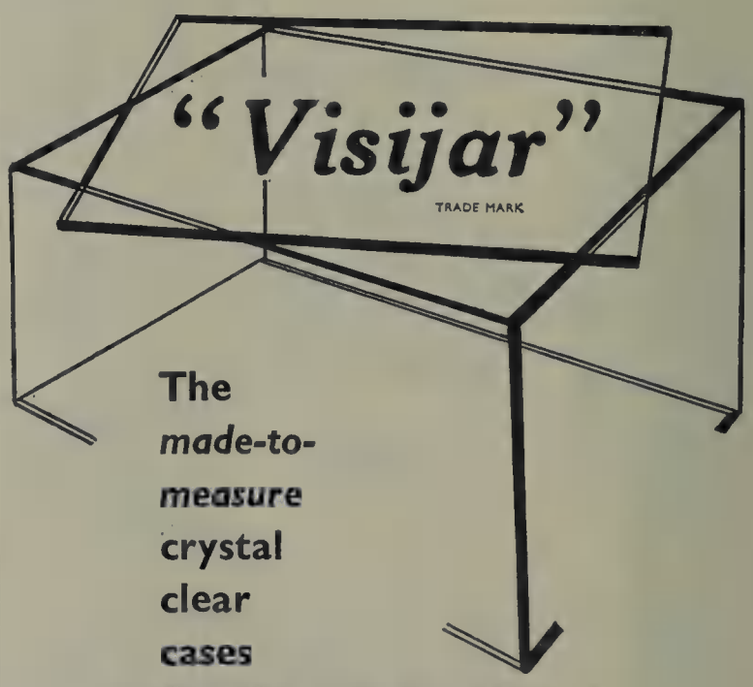

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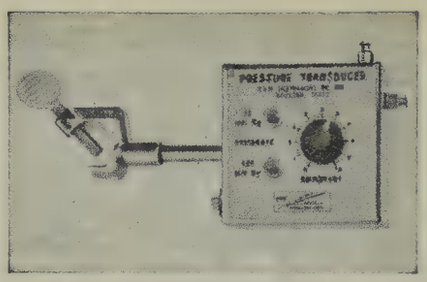

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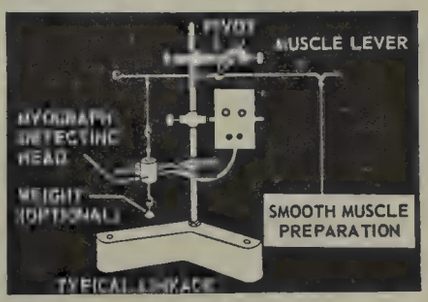

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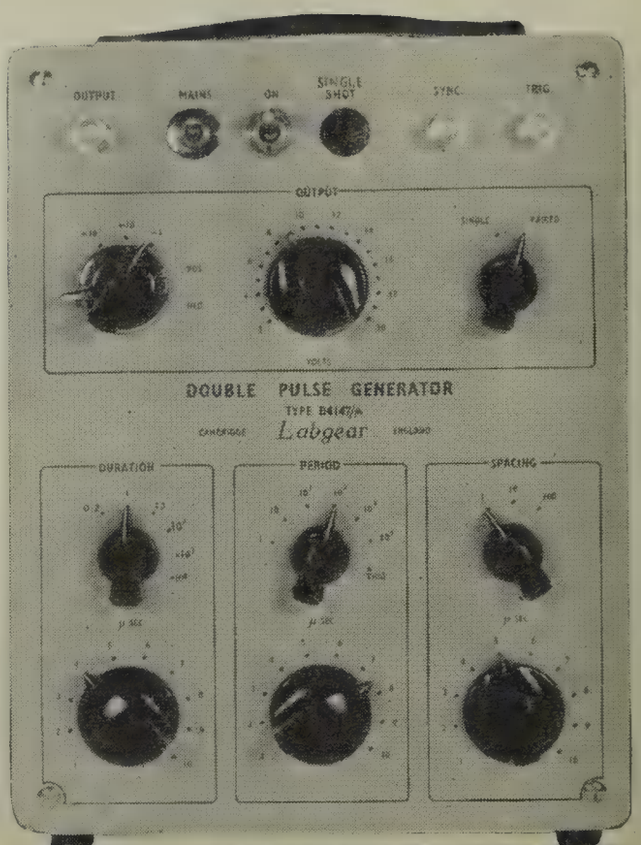

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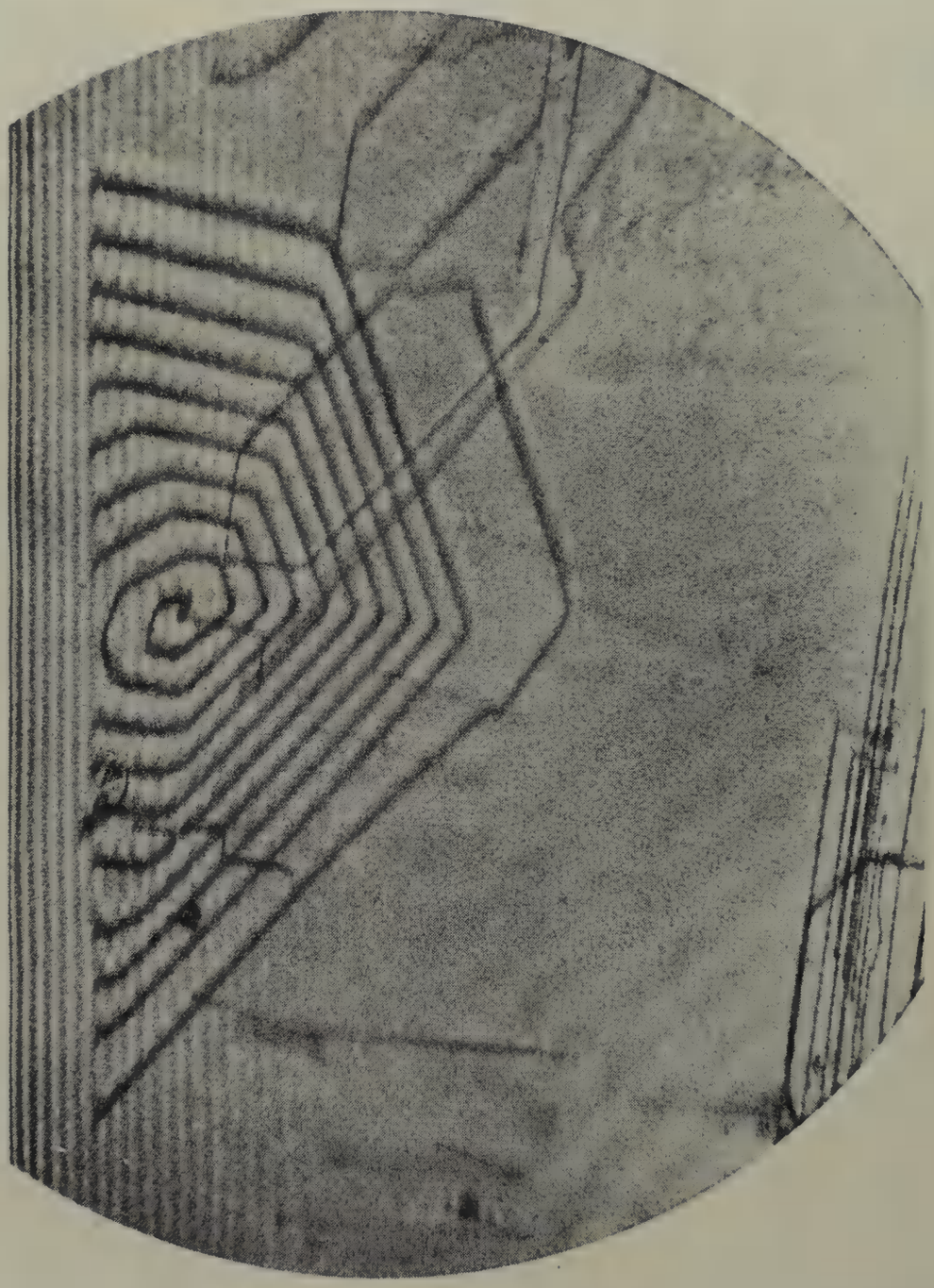

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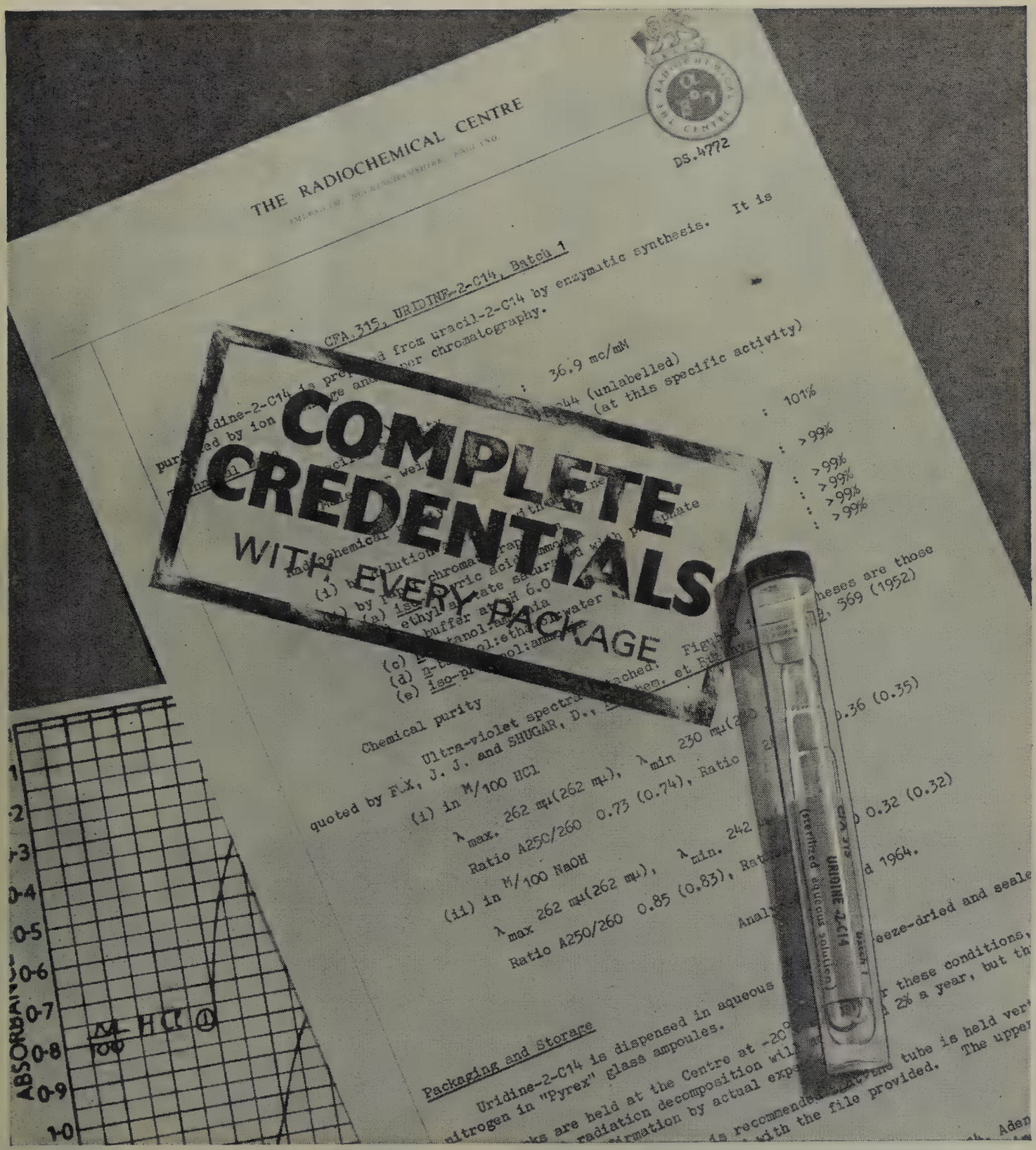

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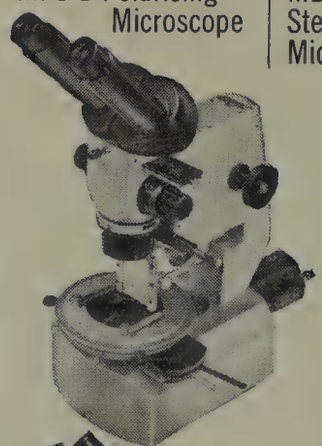

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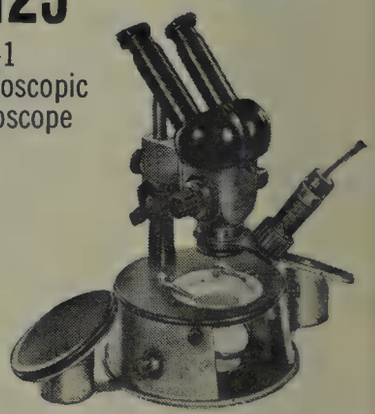

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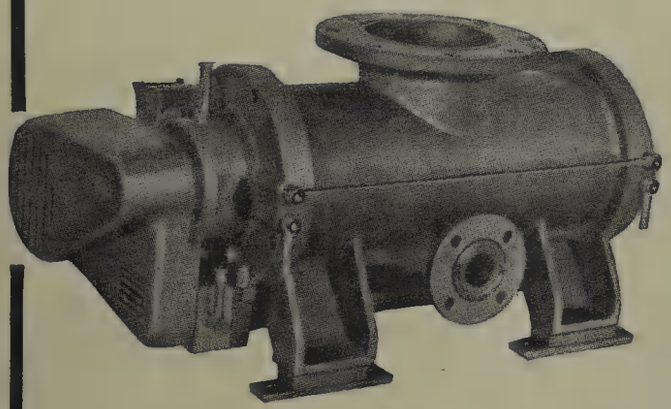

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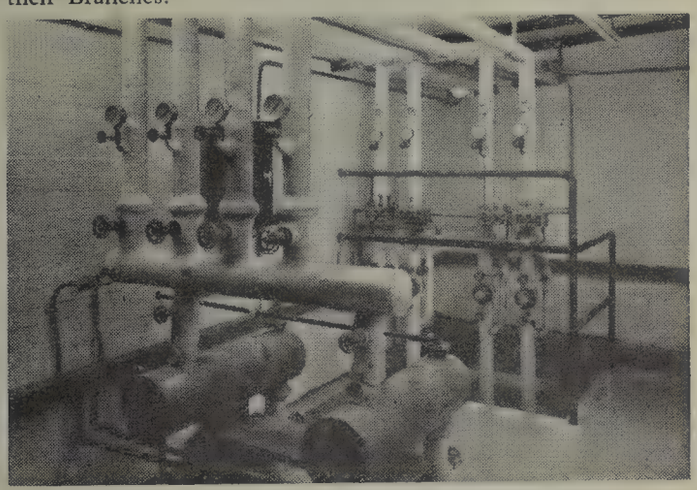

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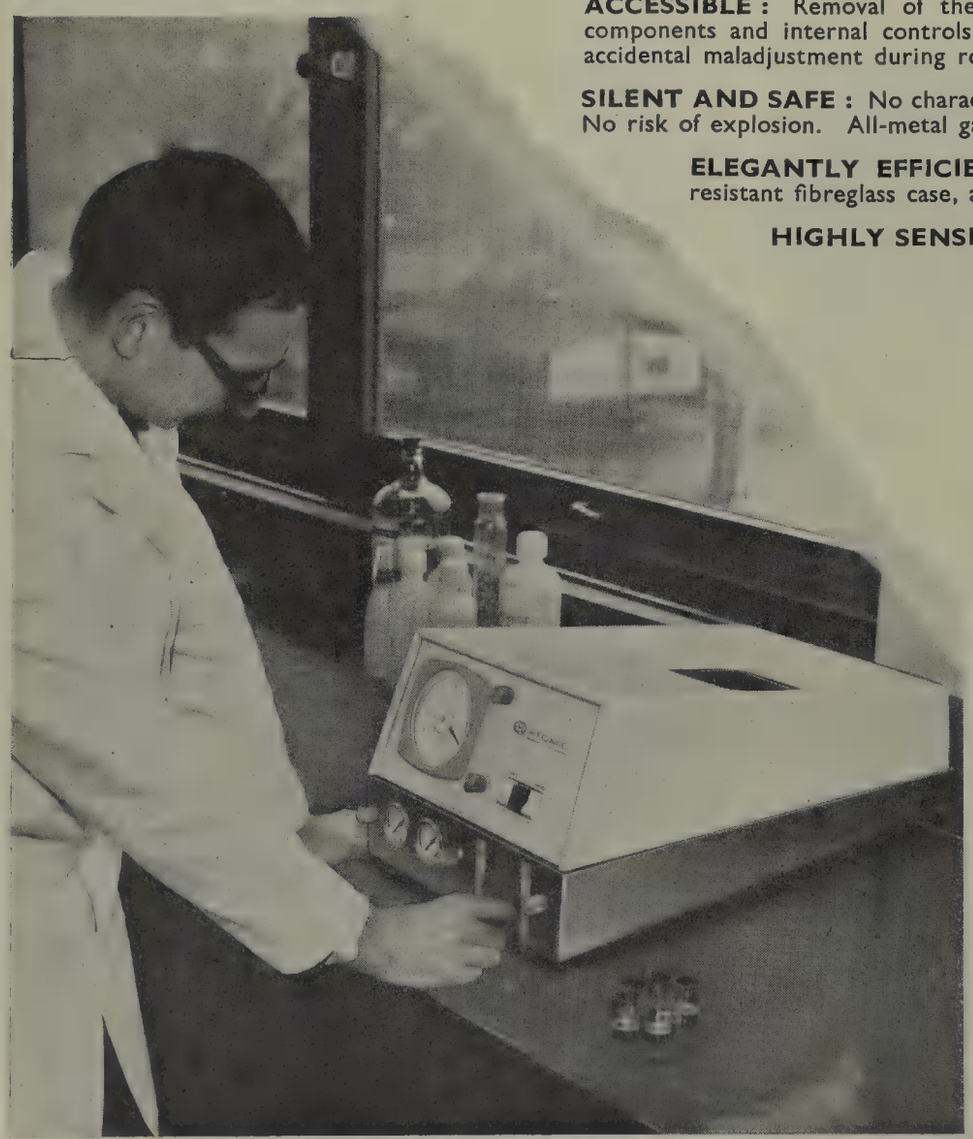

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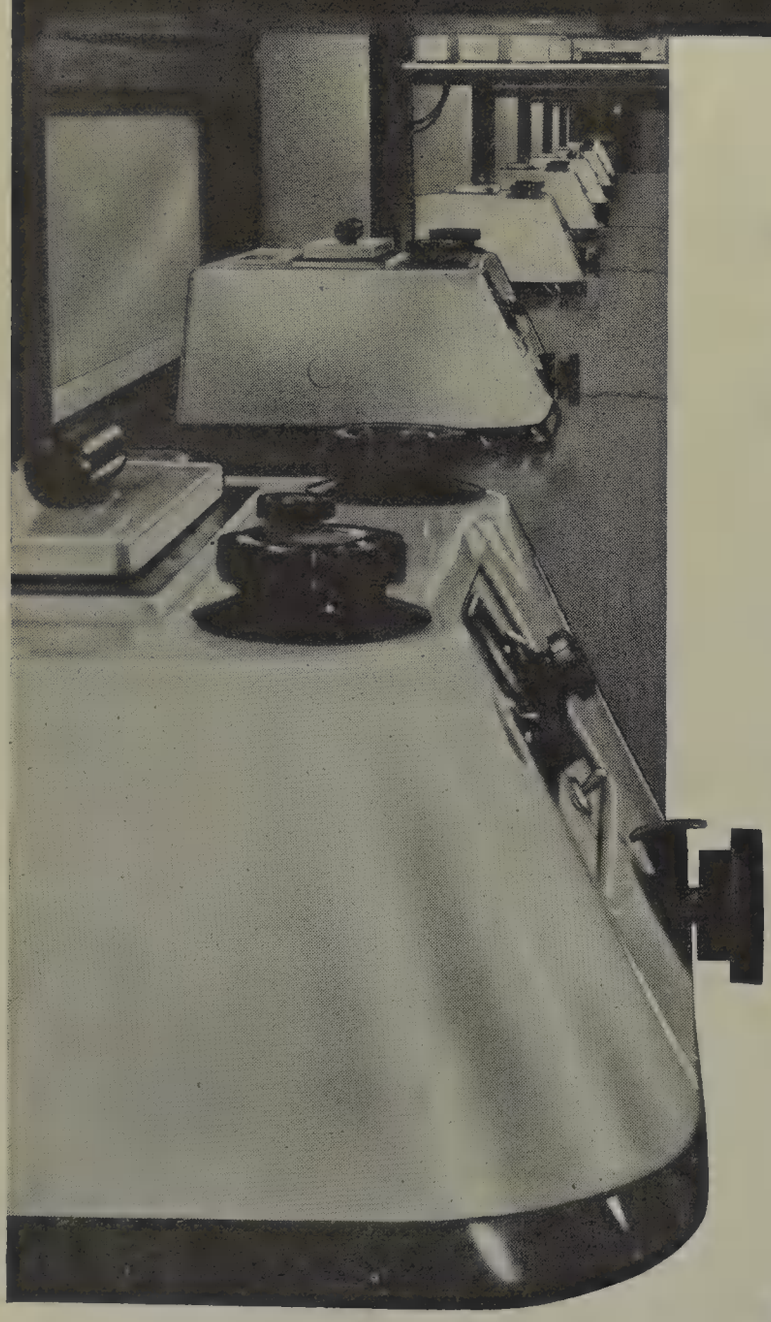

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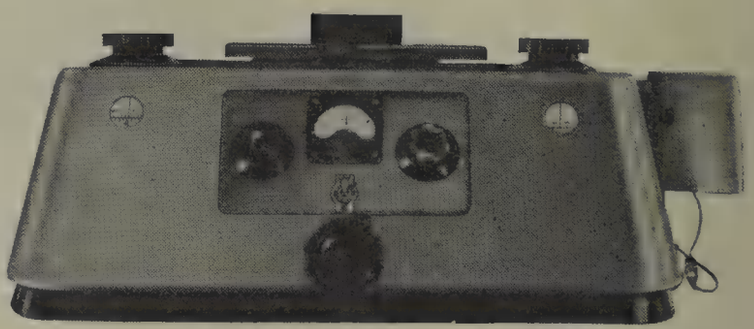

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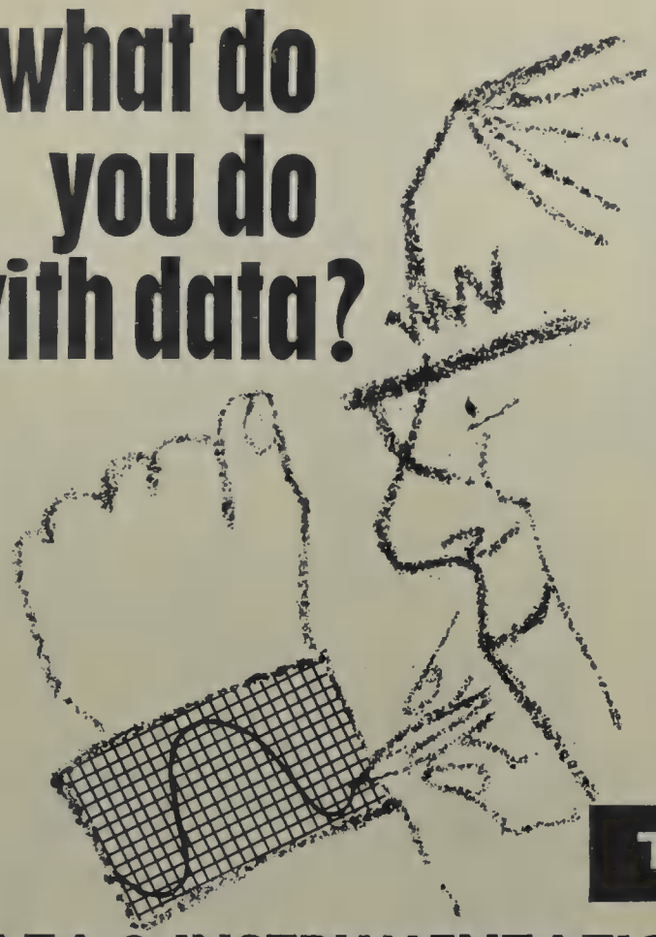

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SCIENTIFIC AND TECHNOLOGICAL INFORMATION IN PARLIAMENT

AT a meeting in June 1964, the Parliamentary and Scientific Committee agreed to set up a sub-committee, consisting of the Parliamentary members of its Steering Committee, to consider whether there was need for improved methods by which Members of Parliament could quickly obtain information from scientists about matters likely to be raised in Parliament. Moreover, the sub-committee was to consider what could be done to improve existing machinery to ensure that Parliament could establish more effective control over scientific and technological policy. For the purposes of this enquiry, the sub-committee co-opted Sir Harry Legge-Bourke and Dr. Jeremy Bray. An interim report entitled "Parliament and Science", dealing solely with the means for ensuring more effective Parliamentary control over scientific and technological policy, has now been issued. This is included, together with a memorandum by Dr. Bray on "Financial Procedure", as an appendix to the fourth report from the Select Committee on Procedure for the Session 1964–65*.

Although in the report from the Select Committee on Procedure this memorandum constitutes the chief interest to the scientist, it is not the sole feature of interest. The report as a whole and its four recommendations are of general interest, reflecting the critical problem of the mechanism of advice and informed discussion in general, but it may be as well to deal first with this report from the Parliamentary and Scientific Committee. Its sub-committee fully recognized that science and technology are both means and objects of policy, and that, besides their responsibility for implementing any policy adopted, experts could point out the probable effects of alternative choices. The sub-committee also recognized that, if Parliament decided to improve the present system of Parliamentary control of policy and administration in the scientific field, for example, by means of a 'select committee', it would have to define its order of reference so as to try to avoid becoming involved in policy decisions.

The sub-committee had before it a paper written by the chairman of the Parliamentary and Scientific Committee, Mr. Austen Albu, entitled "The Member of Parliament, the Executive and Scientific Policy", published in *Minerva* in 1963, to which reference has already been made in *Nature* (**201**, 955; 1964), as well as a pamphlet *Change or Decay* written by a group of Conservative Members of Parliament. However, the sub-committee decided to restrict itself to the means by which Parliament could be better informed on research and development in the civil field undertaken with public funds and in support of public policy. The considerations already noted led it to recommend the establishment of a select committee to examine, with the view of informing the House on their work and future, the annual reports of such bodies as the Research Councils, the Atomic Energy Authority, the National Research Development Corporation, and also the activities of scientific research groups and establishments in Government departments. It also recommended that this committee should, from time to time, make *ad hoc* enquiries into scientific and technological matters which are under consideration as a basis for legislation or the formulation of policy.

The advantage of this proposal would be that Members of Parliament would hear something of the differing scientific and technical views which were under consideration in the department concerned. This would make for more informed debates and should also assist in differentiating technical from political issues. Admittedly reference to a select committee should be sparingly used, but the sub-committee hoped that a practice would develop of referring legislation involving scientific matters to this committee before or during its passage through Parliament. The sub-committee recommended that the select committee should consist of thirteen members (five to constitute a quorum), and that it should have the power to send for persons, papers and records. It did not consider that the select committee should be provided with expert staff but rather that experts should be called, when required, as witnesses. It was hoped that an expanded library and reference service, with scientific staff, would also be available to assist the proposed committee.

This latter point is strongly urged in a memorandum by the Study of Parliament Group, which is a private group of university teachers and officers of both Houses of Parliament which has been meeting since the summer of 1964, under the chairmanship of Sir Edward Fellowes, for the serious study of the working of Parliament. The memorandum concentrates on the procedure on public Bills and on the scrutiny and investigation of policy and administration; it emphasizes that Parliamentary scrutiny of the Executive is fundamental to the whole question of Parliamentary reform. The main task of the Government is to govern, and the main tasks of Parliament are to sustain the Government and to criticize its policies and actions.

The memorandum firmly asserts that Parliamentary control means influence, not direct power; advice, not command; criticism, not obstruction; scrutiny, not initiative; and publicity, not secrecy. The Group believes that strong government needs critical opposition; it can benefit from such criticism and stand up to it. Accordingly the Group suggests a greater degree of specialization by Members of Parliament and the Committees through which, increasingly, they should work. It is in this context that it points to the implications for the research and information services available to Parliament. Changes in and expansion of the services provided by the Library could be as important as any procedural changes in the basic task of creating or restoring adequate supervision by Parliament of the activities of Government. Present difficulties in this respect are, in the view of the Group, unlikely to be resolved unless Parliament is willing some time to look at the problem as a whole and consider how procedure, research and information, facilities and accommodation, form part of an integrated system so that changes in one, voluntary or involuntary, must lead to changes in others.

The essential aspect of any comprehensive proposal for enabling the House of Commons to perform its crucial role in scrutiny and investigation of policy and administration is the provision of adequate research and information facilities for both committees and individual Members.

* Fourth Report from the Select Committee on Procedure, together with the Proceedings of the Committee Relating to the Report, Minutes of Evidence and Appendices, Session 1964–65. Pp. xxiii + 148. (London: H.M.S.O., 1965.) 15*s.* net.

Only a properly developed Library with an expanded research department could provide access to the information necessary to Members for the informed discharge of their Parliamentary responsibilities, and the Group believes that the present research services of the Library could and should be improved. It has, in fact, prepared a report on the provision of research and information services which it hopes to submit to any committee established to consider this problem.

The Select Committee on Procedure did not, in the present report, pursue this question, but its four recommendations give substantial effect to the recommendations of the Parliamentary and Scientific Committee and to the proposals of the Study of Parliament Group for Specialist Committees of Advice and Scrutiny. Five such committees were suggested by the Group for initial experiment, covering scientific development; prevention and punishment of crime; machinery of national, regional and local government and administration; housing, building and land use; and the social services. The Select Committee on Procedure recommends the establishment, as a development of the present Estimates Committee, of a new select committee "to examine how the departments of State carry out their responsibilities and to consider their Estimates of Expenditure and Reports". The new committee should function through sub-committees specializing in the various spheres of Government activity and should be able to employ specialist assistance. There should be two clerks supervising the work of the Committee and one full-time clerk to each sub-committee. The power of select committees to adjourn from place to place should include the power to travel abroad, with the leave of the House, when investigations require it.

In making these recommendations, the Select Committee was obviously influenced by the experience gained with the Select Committee on the Nationalized Industries. It was also noticeably influenced by views expressed by the Estimates Committee, which is precluded by its terms of reference from investigating long-term proposals and prospects for expenditure in the various fields or from examining the administrative policy of Government departments apart from considerations of economy alone. It is not the wish of the Select Committee on Procedure that the specialist committees proposed should become involved in matters of political controversy. Certainly, it is well aware that it is not easy to distinguish between what are questions of policy and what are not, but it believes that the Select Committee on the Nationalized Industries has demonstrated what is possible in a field that is politically highly sensitive. The Committee was anxious also to retain the experience and method of work of the Estimates Committee, and for this reason recommended a series of sub-committees operating through a co-ordinating committee rather than a series of independent committees.

While the recommendations do not strictly follow those of the Parliamentary and Scientific Committee, running more on departmental lines than those of the Study of Parliament Group, it should still be possible for scientific matters to be examined in a context such as the examination of the annual reports of the Research Councils and other bodies would provide—as suggested by the former Committee. The idea of specialist committees has had powerful advocates in recent years, though Mr. Albu did not give it strong support in his paper on "The Member of Parliament, the Executive and Scientific Policy". The more limited proposal of the Parliamentary and Scientific Committee might yet command wider support among those who recognize the vital importance of informed discussion of scientific and technological matters in forming public policy. Curiously enough, the recommendation drew no comment from Dr. Horace King in his statement before the Committee. However, he did affirm that he was one who thought that the power of the Executive was growing and ought to be diminished. For all that he wished to preserve, so far as possible, Parliament's historic and traditional features, he was concerned above all to promote its efficiency and would remove anything that impaired it.

Mr. Albu at least recognized the growing demand, especially among younger Members, for a properly equipped research and information service, staffed with men and women who had been educated in a broad range of subjects, including the scientific and technological. The observations of the Study of Parliament Group on this point are among the most pertinent in the whole report and deserve further attention by the Parliamentary and Scientific Committee itself. Adequate library research and information services are indeed a key factor in informed discussion in Parliament. The Parliamentary and Scientific Committee should be able to do something to see that the observations of the Study Group, which includes such authorities as Prof. M. Beloff, Prof. P. Bromhead, Prof. A. H. Hansen, Prof. W. J. M. Mackenzie, Prof. W. A. Robson, Prof. H. Wiseman, Dr. D. N. Chester and Dr. B. Crick, are duly noted and that the committee suggested to look into this whole question is duly established. That may not be the whole solution, but it is the essential factor. Unless such a step is taken, any system of specialist committees is unlikely to work really effectively. The fourth report from the Select Committee on Procedure provides evidence which should be noted and used by all who recognize the value of informed public discussion in the formation of public policy—above all where scientific and technical factors are involved.

THE MAMMALS

Mammals of the World

By E. P. Walker, F. Warnick, K. I. Lange, H. E. Uible, S. E. Hamlet, M. A. Davis and P. F. Wright. Vol. 1, Pp. xlviii + 644. Vol. 2, Pp. viii + 1,500. Vol. 3, Classified Bibliography, Pp. xii + 769. (London: Oxford University Press; Baltimore: Johns Hopkins Press, 1964.) 300*s*. per set.

THE mammalian fauna of the world at present consists of something between 4,000 and 4,500 living species divided into about a thousand genera—1,044 are recognized as valid in this work, which gives an illustration of all but four of them. The validity of genera as of species is, however, often a matter of opinion that varies widely between the 'splitters' and the 'lumpers' among taxonomists; Walker himself speaks of the "twelve to fifteen thousand species" of mammals, a generous total even for a splitter.

Ernest P. Walker has been amassing the material for these books for more than thirty years, twenty-seven of them while he held the post of assistant director of the National Zoological Park at Washington, D.C. After his retirement the completion of the work was supported for seven years by funds from the National Institutes of Health, and the publication was sponsored by the New York Zoological Society. As the President of that Society says in the foreword, "The heroic task Mr. Walker set himself was to find and reproduce a photograph of a living representative of every genus. If he has not quite accomplished this, he has missed by the narrowest of margins and only because the genus is too obscure or the

photograph hidden where world-wide enquiries could not find it". He has had perforce to use some photographs of stuffed animals in museum collections, and occasionally to reproduce drawings from original descriptions. The illustrations nevertheless form a unique and extremely valuable gallery, for they have been carefully chosen to convey a "better image of the animals than could be given in a word picture . . . many attractive and appealing photographs have been rejected because they did not show the characteristic form of the mammals to the best advantage". More than one picture is provided to illustrate some of the larger genera, and there are many insets of important anatomical and osteological details.

The illustrations are accompanied by a concise text summarizing all that is known of the members of each genus; for many genera all recorded information is given in a few paragraphs, such is our lack of knowledge. Information, whenever available, is given about the number of species in each genus, the distribution, measurements and weights, coloration, type of body covering, structural peculiarities, habits, food, gestation period, number of young in a litter, economic importance, and the vernacular as well as scientific names. A page or two of introductory text is also given to each order and each family. A useful feature is the listing of museums holding specimens of mammals that are very rare in collections. A table showing the world distribution of the genera of recent mammals occupies twenty-four pages of introductory matter and, by means of a simple regional code and a few footnotes, makes much useful information available at a glance. The text and illustrations together with a selected bibliography of about 4,500 titles occupy the first two volumes; the third volume, which is available separately for the more specialized worker contains a catholic bibliography of about 50,000 titles, containing many references of a popular character as well as those of serious scientific content. The pagination runs straight through Volumes 1 and 2, and an index to the whole work appears at the end of both volumes; the end-papers carry scales for the comparison of metric and U.S. units of measurements.

The naturalist's first feeling on opening these volumes is one of surprise and delight at their completeness and comprehensiveness; his next, one of amazement that anyone should have contemplated undertaking such a staggering task, still more that he should have finished it successfully. Here are pictures and descriptions of rare and obscure mammals that hitherto had been no more than scientific names in fauna lists—often not even that—as well as pithy summaries of the biology of more familiar forms. It is of equal value to the general naturalist as a review of the mammalian fauna of the world, as it is as a reference book and guide to the literature for the specialist.

Although no one has approached the author's diligence in searching the literature, seeking information by correspondence throughout the world, in personal observation and study, and in developing a high degree of skill in photography, it would be too much to expect human endeavour to avoid occasional inaccuracies in a work of such great size. The state of Mr. Walker's health compelled him to pass some of the editorial burden to his colleagues during the last four years of the marathon, and he handsomely acknowledges their help for doing the "work that I should have done, but my declining energy prevented me from doing my part". There are, inevitably, some misprints, especially in unfamiliar geographical names, and such errors as "8 vol." for "8vo." in the bibliography. A few more serious errors have crept in, especially in the sections dealing with those orders of mammals with which the author presumably had less personal experience, so that he had to rely on others with consequent occasional misunderstanding. As examples one may mention the photograph of young seals on p. 1302, which, according to the caption, are common or harbour seals, *Phoca vitulina*, but are unmistakably the young of the harp seal *Pagophilus groenlandicus*; or the photographs on p. 1129 labelled "Common or Harbor Porpoises (*Phocaena phocoena*)" which plainly represent the bottle-nosed dolphin, *Tursiops truncatus*—the error here may have arisen from the unfortunate difference between the American and English use of the names "porpoise" and "dolphin". Mr. Walker himself realizes that the devil is ever at hand to frustrate man's striving for perfection, and asks readers who find errors or who know of better photographs or additional facts to communicate with the publishers so that improvements can be made in a future edition.

It is, however, really ungenerous to point out the few blemishes in so splendid an achievement, and all naturalists, especially those who concentrate their studies on the mammals, are deeply grateful to Mr. Walker and his team for consolidating the foundations on which their work is based. The three volumes are not cheap but they are well worth their price, and considering that they contain nearly 1,800 half-tone illustrations it is surprising that they do not cost more; the typography, paper and binding are excellent.

This is one of the major contributions of the century to the study of mammalian biology. The author and his colleagues are to be congratulated on their unremitting labours, and the National Institutes of Health, the New York Zoological Society and the Johns Hopkins Press all gain the highest credit for the parts they played in bringing them to fruition.

L. Harrison Matthews

SALT TOLERANCE IN PLANTS

Physiological Basis of Salt Tolerance of Plants

(As Affected by Various Types of Salinity.) By B. P. Strogonov. Translated from the Russian by Prof. A. Poljakoff-Mayber and Dr. S. M. Mayer. Pp. 279. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 88*s*.

At a time when more and more countries are embarking on ever-increasing schemes for the irrigation of vast areas of arid land, it requires no effort of imagination to appreciate the potential usefulness and relevance of a book dealing with the physiological basis of salt tolerance in plants.

Physiological Basis of Salt Tolerance of Plants was first published in the U.S.S.R. in 1962, and we are indebted for its rapid translation into particularly lucid and easily readable English to Prof. Poljakoff-Mayber and Dr. Mayer working under the auspices of the Israel Program for Scientific Translations. In a note at the beginning it is stated that the translation was undertaken because this was the first attempt to summarize in one book the information available in this field; it contained a great deal of factual information not readily available to Western scientists, and presented a number of ideas not previously adequately stressed in work on salt tolerance. There can be little doubt that these claims are fully justified by the subject-matter of the book.

In the first three chapters, Dr. Strogonov reviews the present state of the physiology of salt tolerance, the mode of action of salts on the plant, and theoretical considerations of experimental results, and this section provides a valuable and stimulating introduction to the literature, especially that from the U.S.S.R. on this subject. In the following eight chapters a series of subjects is dealt with one by one, each directly related to the growth of plants under saline conditions. These include the effect of salinity on development and anatomical structure; water relations and adaptation; nitrogen metabolism, and effects on the soil microflora, and a contribution on practical means for increasing salt tolerance of plants. In each of these chapters an extensive review-type introduction is followed by a section in which the results of experiments performed by Dr. Strogonov with cotton are presented by means of

tables and illustrations, and finally a concise summary of these results is provided. This method of presentation is not entirely successful since it provides the reader with a very large bulk of rather indigestible experimental results, and, owing to the absence of a discussion of the results by the author, considerable effort is required to correlate the experimental results reported with the information detailed in the introductory review of the literature.

Dr. Strogonov stresses that the book is concerned not so much with observations of conditions arising from salinity as with the mechanism of the action of excess salts on plant growth, and the specific effect of the type of salinization of the substrate. In particular, the importance of distinguishing between osmotic effects and toxic effects is emphasized. It is suggested that the toxic effects are due not directly to the effects of the salts themselves but to interference with the normal metabolic processes of the plant resulting in the accumulation of toxic substances such as ammonia, hydrogen peroxide or putrescine.

The value of the book is wantonly reduced by the complete absence of any sort of index, and also by the quite appalling quality of the illustrations. Nevertheless it does provide a useful and provoking review of the present knowledge on the subject, and, to the Western scientist, an excellent bibliography of a large body of inaccessible Russian work. It should provide a considerable stimulus to a branch of plant physiology which, in view of its outstanding practical importance, has been sadly neglected in recent years.

P. A. Thompson

RESEARCH IN PHOTOSYNTHESIS

Photosynthetic Mechanisms of Green Plants

(Papers presented at a Symposium sponsored by the Committee on Photobiology of the National Academy of Sciences–National Research Council, October 14–18, 1963.) (Publications No. 1145.) Pp. ix + 766. (Washington, D.C. : National Academy of Sciences–National Research Council, 1963.) n.p.

THIS volume contains the papers presented at a symposium sponsored by the Committee on Photobiology of the National Academy of Sciences–National Research Council, in October 1963.

It provides a wide, if uneven, coverage of the field of photosynthesis to that date, and is divided into ten main sections, including spectroscopic and fluorescence studies; biochemical studies of electron transport paths, and of isolated electron carriers; enhancement studies; respiration, photosynthesis and hydrogen metabolism; functions of pigments; chloroplast structure, mechanism of phosphorylation; carbon metabolism.

The volume contains seventy-five papers, of ten pages average length. Not unexpectedly, these differ widely in content, in presentation and in style. Some authors make a real attempt to review work in their particular laboratories, and to synthesize a picture of the mechanism of photosynthesis from the confusion of experimental data, but others present only isolated experimental data, thinly or thickly spread. Inevitably the volume lacks cohesion, and would have been better for a more uniform presentation. Very little of it is easy reading, but one feels that to include papers in which the text, the figures, and the figure legends are each in separate sections is asking too much of any reader. Quick publication is an important aim, but one feels that the volume would have been much more valuable had more rigid instructions for presentation been enforced.

The volume includes useful discussion of the nature of the long-wave absorbing pigments, and of the trapping centre of System I, which also serves to direct attention to our ignorance of System II. Work on biochemical mutants and their contribution to our present picture of photosynthesis is well covered. The volume emphasizes how a wide range of techniques and experimental approaches combine to give a reasonably coherent, if incomplete, view of the process, and much of the discussion centres around this picture of two photo-systems acting in co-operation with one another. The nature of the biochemical events set in motion by the absorption of quanta in each photo-system, and the mechanisms of the co-operation between them, provide much of the discussion and reflect the amount of effort at present devoted to these aspects of the subject.

By contrast, there is very little discussion of new information on the processes of energy transfer from the site of the initial absorption of a quantum to the active centre from which the subsequent biochemical changes arise. Nor is there evidence presented on the transformation of excitation energy in oxidation–reduction reactions at the trapping centre—another of the major unsolved problems in the field. Thus the book, both by its content and its omissions, presents a useful picture of the present state of the subject.

There is a seven-page editorial summary, which does something to draw together the wide range of experimental results and of hypotheses, but inevitably, at such a length, this can only highlight the more important points covered, and direct attention to areas of strong interest or extreme ignorance. One would like to read a much more complete, critical assessment of the papers presented. Thus as a review this book is less satisfactory than other recent reviews of the field, and its value is rather as a source book. Unfortunately for this purpose an index becomes a prime necessity and this is entirely lacking—doubtless a sacrifice to rapid publication. The book is essential reading for those working in the field of photosynthesis or in related fields, but it is a book for those already familiar with the literature; it is too heavy going to be in any way an introduction to the subject even for advanced students. With these limitations, it provides a valuable collection of papers covering a range of work which is otherwise widely scattered, and will be useful as such.

E. A. C. MacRobbie

INDIAN TIMBERS

Indian Woods

Their Identification, Properties and Uses. Vol. 2: Linaceae to Moringaceae. Pp. x + 386 + plates 31–63. (Delhi: Manager of Publications, 1963.) Rs. 32–50 nP.; 75*s*. 10*d*.; 11.70 dollars.

THIS is the second of a series of volumes (the first of which was published in 1958) describing the timbers of India which are already commercially important or which are likely to become so as timber supplies run short. In the present volume the woods of 263 species are described, and these belong to 23 families ranging from Linaceae to Moringaceae. Descriptions and editing are mainly by S. S. Ghosh, K. R. Rao and S. K. Purkayastha, who have been supported by a team of assistants and specialist colleagues. Dr. K. A. Chowdhury, the senior joint author of the first volume, has revised the drafts of twenty of the present family descriptions and has also helped to select the photomicrographs.

The present volume is in the same style as the first. The reader is told under each family the number of genera and species of which it consists, and there are notes on economic plant products. Then follows a description, based mostly on naked eye or lens characters, of the gross structure of the timber of each of the species selected for inclusion in the book. Each description of the structure is followed by notes on durability, liability to attack by insect or fungus, working qualities, availability of supplies and, finally, uses. Reference numbers of the wood specimens on which the descriptions are based are given, and at the end of each family description there is a biblio-

graphy. With some of the larger families there are keys to the identification of the genera based on wood structure.

At the end of the book there are four appendixes dealing, respectively, with mechanical properties, classification of woods according to anatomical structure, classification of woods according to uses and, finally, recent name changes. In the second of these, the genera are very usefully listed under the diagnostic characters by which they can most easily be identified. It seems a pity that the name changes in Appendix IV are not covered under "Synonymy" in the main body of the text. There are 197 photomicrographs, mostly of excellent quality, showing the end-grain appearance of the different woods, and the book closes with separate indexes to (i) scientific names, and (ii) trade and common names.

This excellent book deserves a much better cover, for it will undoubtedly have to stand up to the hard wear that frequent consultation entails. C. R. METCALFE

TAXONOMIC AND EXPERIMENTAL BACTERIOLOGY

Manual for the Identification of Medical Bacteria

By S. T. Cowan and the late K. J. Steel. Pp. x+217. (Cambridge: At the University Press, 1965.) 50*s*.

Theory and Practice in Experimental Bacteria

By G. G. Meynell and Elinor Meynell. Pp. xii+288+2 plates. (London: Cambridge University Press, 1965.) 50*s*. net; 9.50 dollars.

THE demand for diagnostic tables for the common medical bacteria by these authors was so great among clinical bacteriologists and others that it was inevitable that they should produce a book on the subject. It is therefore most regrettable that K. J. Steel, who contributed much to this work, did not live to see the proofs.

The *Manual* is clear and well produced, written with authority and an insight which at times is almost embarrassing; for who has not at one time or another been tempted to create a new species rather than carry out the necessary work to put an organism into its right place in an existing species. The book is centred on the two- and sometimes three-stage tables, which are easily read and clearly understandable to the bacteriologist. These do not, however, by themselves characterize organisms, but as the authors state, they focus attention on the most valuable of taxonomic tests. The early chapters are devoted to classification and nomenclature, the importance of obtaining pure cultures, and a useful chapter is included on the much-vexed problems associated with media preparation and sterilization. There follows a chapter on characterization in which the primary and secondary tests for the first and second stage diagnostic tables are discussed. The authors then get down to describing characters of Gram-positive and Gram-negative bacteria, and include what they term "minidefinitions" which give the main characters of each organism succinctly and precisely. These should be very helpful to harassed clinical bacteriologists who wish to refresh their memories as briefly as possible. Following these chapters is one on refractory organisms of uncertain characterization; and a short chapter on taxonomic implications (for those interested) ends the discursive part of the book.

There are seven appendixes, consisting of laboratory notes covering instructions for media preparation and control, staining reagents, characterization tests including rapid methods some of which, it is claimed, are sensitive and give more clear-cut results than the standard characterization tests. Finally, there is a list of recommended strains of test organisms with conditions for their maintenance; and the book ends with a comprehensive list of references.

The *Manual* will undoubtedly be popularly received in most diagnostic laboratories, and this must be due in no small measure to the realization by the authors of the different points of view between the taxonomist and the clinical bacteriologist, or the taxonomist and the epidemiologist. The majority of workers who will use this book will undoubtedly have ideas as to the probable causal organisms to be isolated from a known specimen. Whether their assessment is correct or not, they can with value use the progressive approach to identification indicated by the tables, and probably arrive at a correct diagnosis. Isolation of pure cultures of organisms from clinical specimens is the prime requirement, and it seems a pity that more comprehensive advice on this problem, and on the use of enrichment media, was not included within the stout covers of this admirable handbook.

Experimental microbiologists and research students will find themselves well served by the efforts of Meynell and Meynell in a book which is packed with information about measurement, both quantal and quantitative, of micro-organisms, and which does not contain one single dull or uninteresting page. This is a fascinating guide to theoretical principles underlining the significance and importance of bacteriological experimentation, and can be approached with minimal understanding of simple statistical terms.

The book commences with a chapter on measurement of bacterial mass and number, neatly describing the various methods in use and showing clearly that in estimation of the precision of viable counts the presence of sampling errors lessens the effect of technical error. The section on quantitative measurement of bacterial growth could be read with profit by undergraduate students of bacteriology.

The next four chapters deal with culture media; oxidation–reduction potential and anaerobiosis; the kinetics of sterilization, including sections on chemical disinfection and filtration; and microscopy. The penultimate chapter, dealing with quantal responses, Poisson distribution and normal and log-normal distributions, is supremely clearly written, and with well-chosen examples makes relative simplicity out of what, to a large number of biologists, are complex and difficult subjects. The book ends with a chapter on miscellaneous techniques.

Not the least contribution of this book is the inclusion of a variety of most useful tables which would be of considerable value in any microbiology laboratory.

D. ALLAN

SKIN AND ITS DISEASES

The Epidermis

Edited by William Montagna and Walter C. Lobitz, jun. Pp. xx+649. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 107*s*. 6*d*.

FOR a variety of reasons which are familiar to most scientific workers, the published proceedings of symposia rarely contain any information which is not available elsewhere. They are, however, sometimes of value in two ways. First, they may bring specialized information to the notice of a reader from an allied field of research with which he is not familiar because of its publication in journals not immediately available to him. Secondly, symposia may be used by investigators to take a broader look at current thought in their own field. Both these aspects make their appeal on reading the papers presented at a symposium on "The Epidermis" which was sponsored by the Division of Dermatology of the University of California. It was made possible by a grant from the U.S. Public Health Service, the advisory committees of which felt the need for a series of such meetings on the biology of the skin.

The volume is dedicated to the late Prof. Stephen Rothman, a pioneer dermatological investigator, whose first chapter on "Keratinization in Historical Perspective"

sets the high standards to follow. The book is large but contains a wealth of basic dermatological thought and fundamental information. The chapters range widely over embryology, keratinization, ultrastructure and histochemistry, carbohydrate, nucleic acid, sterol and lipid metabolism, permeability and blister formation. Many of them are contributed by the acknowledged experts in each field whether from the United States, Europe or Australia.

Philippe Sengel (Paris) describes his elegant experiments which have thrown so much light on the differentiation of cutaneous appendages in the embryo, and Dame Honor Fell summarizes the present studies on keratinization in organ culture of skin being carried out at Strangeways Research Laboratory. Mercer (Canberra), Brody (Stockholm) and Swanbeck (Stockholm) each give excellent accounts of their own work on the ultrastructure of the components of the horny layer in normal skin, and the deviations which characterize psoriasis. Kligman's (Philadelphia) chapter on the biology of the stratum corneum is especially readable, not only because of his own distinctive style of writing but also because he has directed attention to some of the more neglected biological phenomena in the epidermis. Epidermal cells in the stratum corneum become very much more cohesive to one another than in the basal, Malpighian or granular layers, and yet this cohesion is lost at the surface in such a way that the fully keratinized cells are shed in small flakes which are not normally perceived. An understanding of the mechanism by which such a change occurs should lead to valuable knowledge of many scaly dermatoses.

The organizing committee of this symposium were fortunate to succeed in 'capturing' so many outstanding scientists actively working on the biology of the skin. Drs. Montagna and Lobitz and the Academic Press have produced a volume which is a credit to the participants. There is an author index of the references used in all the papers, and a subject index. Typographical errors are very rare indeed. "Bellberg" for "Bettley" is repeated several times. For a book of this quality it is not overpriced.

C. D. Calnan

ATOMIC AND MOLECULAR SPECTROSCOPY

Spectroscopy and Molecular Structure

By Gerald W. King. Pp. xiv+482. (London: Holt, Rinehart and Winston, 1964.) 86*s*.

EXCELLENT advanced monographs on many different aspects of atomic and molecular spectroscopy are available, and articles in, for example, *Annual Reviews of Physical Chemistry* and *Advances in Spectroscopy*, enable research workers to keep abreast of present development. There is, however, at a different level, a need for an introductory text-book suitable for senior undergraduate or postgraduate teaching in chemistry or in physics. Dr. King's book fills some of the gaps admirably.

A very short introduction on spectroscopic technique is followed by chapters on wave-mechanics and the hydrogen atom, many electron atoms, diatomic molecules, the symmetries, rotations and vibrations of polyatomic molecules, and concludes with a discussion of the electronic spectra of polyatomic molecules and appendixes on operators, selection rules, character tables and physical constants. Each chapter has a short bibliography, and a collection of illustrative problems, some of which are very searching. Mathematical techniques are introduced progressively throughout the book, so that the later chapters are, reasonably, much more difficult than the early ones. However, the elements of group theory and of matrix algebra, required for the treatment of polyatomic molecules, are introduced both clearly and attractively.

There has necessarily been a rather careful selection of material, so as to limit the book to a convenient length. Microwave spectroscopy is one of the subjects to suffer. Some description of the techniques would have been welcomed by many students, and the measurement of dipole moments and of nuclear quadrupole coupling constants has provided information of such wide interest that it is surprising that these subjects have been omitted. Again, chemists interested in the properties of complex compounds will find the discussion of the energy states of transition metal atoms and ions inadequate. The treatment of dissociation energies is also very brief (it would have been helpful to have included the paper by R. S. Mulliken (*J. Chem. Phys.*, **33**, 247; 1960) in the bibliography at the end of Chap. 6). But, it must be stressed, some selection must be made, and by and large this is a book on the energy-levels of atoms and molecules, and very successful it is too.

The book is well produced and easy to read. The least-successful features of the production are the six half-tone reproductions of spectra. There are very few misprints or mistakes. For example, the statement (p. 75): "Spin-orbit interactions increase rapidly with atomic weight, and are especially large in excited states of heavier atoms" is misleading. These interactions certainly increase rapidly with atomic number, but for a given configuration, say . . . *np*, of a particular atom or ion, the spin-orbit coupling *decreases* rapidly with principal quantum number *n*. Again, p. 211: "The Λ-type splitting seldom exceeds a few hundredths of a wave-number" should be replaced by "The Λ-type splitting *constants* seldom exceed a few hundredths of a wave-number". In the review copy, Fig. 6, 7 (p. 206) was inverted, and there is an obvious error in the printing of problem 8·2 (p. 314).

Nearly half the book is devoted to the energy-levels and spectroscopy of polyatomic molecules, and it is this which is its real strength. This is not an easy subject; but the author has succeeded in developing an account which is both lucid and stimulating. This will be recommended reading for many mathematically minded chemists and physicists, undergraduates and postgraduates alike.

R. F. Barrow

BASIC STATISTICAL TECHNIQUES

Statistics and Experimental Design in Engineering and the Physical Sciences

Vol. 1. Pp. xiv+523. 84*s*. Vol. 2. Pp. ix+399. 87*s*. By Prof. Norman L. Johnson and Prof. Fred C. Leone. (New York and London: John Wiley and Sons, Inc., 1964.)

"THIS book is intended to give students and research workers in science and, particularly, engineering a sound understanding of and facility with basic statistical techniques.

"The material in this book should cover two or possibly three semesters of applied statistics at the upper-division undergraduate level."

These two sentences from the preface indicate the authors' intention. Despite the number of books which aim at fulfilling this function, there is still a need for a good one. The book is long, nearly a thousand pages, and is divided into two volumes. The first volume has three chapters on probability theory describing the properties of the usual distributions, and also some of the distributions which are of practical interest but are often ignored, and chapters on estimation, testing hypotheses, tests of significance, regression and correlation which are not particularly well done and appear in almost any other book of this nature. There are chapters on order statistics and non-parametric methods, both of them clearly written, a long and detailed chapter on control charts, and a balanced chapter on the utilization of prior information. The second volume has a long section, more than two hundred pages, on the analysis of variance and the design

of experiments and a chapter each on sequential analysis, multivariate analysis and sampling. There is an appendix of tables which is comprehensive enough to be useful, and a bibliography of statistical journals and tables. The examples, of which there are a good number well placed in the text, are mostly credible and instructive.

The general character of the book is in line with what the authors take as their definition of statistics, the scientific study of the analysis of quantitative data. It seems to me that by concentrating almost exclusively on the description and analysis of data, the book suffers from a lack of balance. Thus the aim of the chapters on probability theory is to present the distributions which are usually used to describe engineering data, and this is done well enough. But there is no account of why one would expect to use a particular distribution in one class of problems but not in another, nor is there any indication that probability theory might be useful in investigating the structure of a problem. The normal distribution is introduced as being of importance because it fits much of observed data, and is then fitted to some data. Nothing is said about why this should be so. The Poisson distribution is discussed as a useful limiting case of the binomial distribution, but as most of the examples can be described more naturally in terms of the Poisson process, it is a pity the Poisson process is not mentioned. It seems odd that a book should contain an elegant treatment of the non-central χ^2, t and F distributions but have no mention of stochastic processes. Again, the section on the analysis of variance is primarily concerned with the analysis rather than the design of experiments, and little is said of the merits or otherwise of the many designs for which the analysis is presented. The section on multiple linear regression gives several methods for inverting the design matrix, but says nothing on choosing it.

It is difficult to see this book being used as a text-book for engineering students. Most universities in Britain only have short courses in statistics for engineers, and as an introduction to the subject this book is not to be recommended. There is nothing new in the more elementary chapters, which consist mainly of explaining techniques rather than the underlying principles or general usefulness, and a knowledge of statistics based on these chapters alone would probably do more harm than good. To statisticians who do a certain amount of engineering consulting, however, the book might prove valuable, as there are a fair number of topics discussed which many books ignore.

N. E. Day

PACIFIC GEOLOGY

Marine Geology of the Pacific

By Prof. H. W. Menard. (International Series in the Earth Sciences.) Pp. x+271. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1964.) 12.50 dollars; 100*s*.

THE Pacific Ocean comprises nearly half the total area of the surface of the Earth: it is therefore an ambitious project to describe and discuss the geology of the underlying crust in one book. That it is possible at all results from the way in which the subject of marine geology has grown owing to the necessity of conducting operations several miles above the sea-floor. The facility with which rocks can be examined at close range on land led to the growth of geology through hand specimens up to the broader concepts of tectonics and global structures. Submarine geology, on the other hand, has largely developed from the broader studies of bathymetry and geophysical surveys, such as magnetics and seismics, that are necessitated by the inaccessibility of the bottom. Hand-samples are rare, and rigorously controlled sampling is still impossible except in a few special areas.

Prof. Menard has brought together, in a very readable form, nearly all that is known about the Pacific geology and discusses it in terms of the broader concepts of the history of the ocean basins, and continental margins. Inevitably many of the data are mentioned only by reference but this has left the author freer to discuss the relative merits of the different theories presented. As one of the leading authorities on Pacific geology, many of the data have been obtained by Prof. Menard himself and many of the hypotheses discussed have been published by him over the past two decades. However, the book contains new material, especially in the form of summary diagrams, a bathymetric map of the whole Pacific (regrettably split into 14 sections to fit page size) and an admirable physiographic diagram of the north-east Pacific in a folder at the end. This diagram follows the style of those produced by Heezen and Tharp of the Atlantic and Indian Oceans but with the addition of 500-m contours in the basins.

But more important is the development of the new idea of the Darwin rise as an old and foundered mid-ocean rise, the importance of which in the Pacific has been overshadowed by the younger East Pacific Rise. The great east–west linear faults, which are such a feature of the Pacific, are described in detail in Chapter 3, but a discussion of their relevance to crustal deformation is deferred to the later chapter on oceanic rises.

This book is much more than a treatise on Pacific geology. It contains a stimulating review of the features and processes of marine geology in general. Whole chapters are devoted to vulcanism, manganese nodules and turbidity currents, while elsewhere new evaluations are presented of the composition of the seismic second layer, abyssal hills, changes of sea-level and the development of continental margins.

An attractive feature of the book, so often absent in the discussion of geological phenomena, is the order-of-magnitude calculations made about such processes as transportation of sediment through submarine canyons and the origin of sea-water. In the many problems where our knowledge is least sure, Prof. Menard has frankly admitted it and given rein to imaginative speculation that is a challenge to future research.

This book is highly recommended as an up-to-date and well-presented account of marine geology both for the student and for all involved in research in this field.

A. S. Laughton

A CENTURY OF ANTHROPOLOGY

A Hundred Years of Anthropology

By T. K. Penniman. With contributions by Beatrice Blackwood and Dr. J. S. Weiner. Third edition, revised. (Duckworth's 100 Years Series.) Pp. 397. (London: Gerald Duckworth and Co., Ltd., 1965.) 42*s*. net.

THE third edition of Penniman's survey brings up to date the story he first unfolded in 1935. Two extra chapters, by Dr. J. S. Weiner and Miss Beatrice Blackwood, review developments in physical anthropology and in Americanist studies, respectively, over the past thirty years. Readers who can look back to the first appearance of Penniman's classic will remember the admiration it evoked in anthropological circles at that time. The common opinion was that the several branches of anthropology had become too specialized for any one scholar to have competence in all. Pupil and later colleague of R. R. Marett, Dudley Buxton and Henry Balfour, whom he succeeded as curator of the Pitt-Rivers Museum, Penniman showed that it was still possible for an all-round ethnologist to keep abreast of the main trends in all branches of the subject. The extra chapters in the present edition eloquently testify to the end of that epoch.

A history as comprehensive as this could easily degenerate into a catalogue of names, dates and titles of publications—Penniman avoids this by his narrative method. Defining 'anthropology' as the generic science

of man embracing the biological, historical (and therefore archaeological), cultural and social conditions of his existence, he directs attention to the major figures, those whose discoveries and theories set the general patterns of anthropological enquiry for a period of development. Lesser figures are dealt with more summarily in relation to the topics to which they made contributions rather than the issues of principle that were uppermost in their time.

To find Darwin's, Herbert Spencer's and E. B. Tylor's work and influence discussed at length—and, be it added, with a sure sense of what was significant in this for their successors—seems natural enough. But how many of us are aware of the seminal influence exercised by Bastian (1826–1905) during his life-time? Indefatigable traveller and encyclopaedically learned, he is ranked by Penniman with Darwin and Tylor. Bastian is famous for the hypothesis of the "psychic unity of mankind", which he attributed to the universality of a limited number of "elementary ideas". Penniman's account of how he reached his theories, what he really meant by them and how they served to unite the incipiently divergent evolutionary and diffusionist trends of the period is typical of the exposition followed throughout.

Penniman divides the history of anthropology into five main periods: (1) The "Formulary Period", from the Greeks to 1835; (2) The "Convergent Period" (1835–59); (3) The "Constructive Period" (1859–1900); (4) The "Critical Period" (1900–35); (5) The "Period of Convergence and Consolidation", since 1935. For each of the modern periods, each branch of anthropology is treated under a separate rubric, but the details of authors and publications are bound together by keeping common and general tendencies to the fore.

Up to 1935 the story is told with a masterly command of the literature and admirably balanced judgment. To take an instance at random, one would have to search diligently in the anthropological literature of the 'thirties to find comments as judicious and sympathetic as Penniman's on the significance of psycho-analysis for anthropology. The part which traces more recent developments is, inevitably, more sketchy. The excellent index and the careful bibliographical documentation deserve particular commendation.

M. Fortes

THE NON-GUILTY PARTY

Prisoners and their Families

By Pauline Morris. Pp. 327. (London: George Allen and Unwin, Ltd., 1965.) 50*s*.

MOST of us, fortunately, are relatively law-abiding citizens and we are unlikely in the course of our daily work, and may be even during the whole of our lives, to come up against the problems which result from the imprisonment of law-breakers. Occasionally a sensational crime, such as "the great train robbery", and, even more sensationally, the escape from prison of one or more of the convicted train robbers, will attract our attention and give rise to discussion about the penal system and imprisonment as a form of punishment, but it is very doubtful whether most of us will spare a thought even then for the prisoner and his family. On the whole, we prefer to leave the problems of crime and its punishment to the police, the courts and the Home Office (Prison Department) and the few worthy citizens who are prepared to give voluntary service as prison visitors. But are we entitled to ignore so easily the social effects of imprisonment as a method of social punishment, and can we be quite sure that when an offender is punished we do not impose additional punishment on his family?

A remarkably thorough study of the problems encountered by the families of prisoners in Britain has recently been made by Mrs. Pauline Morris, and the conclusions derived as a result of her investigations should make us all think more seriously about the social effects of imprisonment. The great virtue of this book is that it records the results of a truly scientific investigation and yet it is written with warmth, insight and compassion. Despite the appalling misery which Mrs. Morris all too often has to describe, she never allows her emotions to deflect her from the path of strict objectivity and scientific analysis of the data so carefully and systematically collected.

The primary object of the research project was "to elicit facts upon which penologists and administrators might base future policies" and, curious as it may seem, this "is the first attempt in this country to look at the problems of the families of prisoners on a national scale". As a result of previous research undertaken by Mrs. Morris "amongst the recidivist populations of two maximum security prisons" which showed "that a high proportion of men appeared to have severe family problems", a number of hypotheses were put forward to be explored in this new investigation. For example, that "family relationships following upon conviction and imprisonment will follow a pattern set by family relationships which existed before imprisonment", and "the adjustment of the family to imprisonment will vary with the type of offence, and with the extent of previous criminal experience".

A national sample of male married prisoners (837 in all) was selected for interview and permission was sought from each prisoner for his wife (legal or otherwise) to be interviewed at home. In addition, an intensive study of the families of 100 prisoners living in the London area was undertaken. The organizational problems involved in arranging the interviews and carrying them out were enormous, but the care taken and the controls exercised in planning the project and in its execution are of the highest scientific standards. Indeed, for those who still doubt whether scientific methods can be applied to investigations of human and social phenomena, Chapter 11 of this book on "The Design of the Enquiry" should be compulsory reading.

The picture which emerges of the varieties of problems encountered by the families of prisoners makes interesting, and sometimes heartbreaking, reading. Mrs. Morris is to be congratulated on producing such a vivid and living account of the real life problems faced (not always squarely) by real live persons less fortunate (and on occasion less lucky) than most of us. Inevitably there were methodological difficulties which could not always be solved, as, for example, when the 'story' given by the prisoner about his family circumstances differed substantially from that given by his wife. On further investigation "it transpired that the wife shared herself between two 'husbands', both of whom happened to be in gaol at the time of our interview with her. Since this fact was unknown to the interviewer, it was not possible to tell that the information the wife gave related to a different 'husband' from the one whom we actually saw!". Fortunately, there were very few discrepancies of this kind in fact, because of the exacting standards applied by Mrs. Morris to this investigation.

This book undoubtedly makes a significant contribution to our knowledge of a social problem about which all too little was known in the past. It should also provide a firm basis for developing an enlightened penal policy in the future. Mrs. Morris and her colleagues have obviously not given us all the answers we need to solve the difficult problems of punishment, but they have produced an intensely interesting and valuable investigation which can be thoroughly recommended to those (and I hope they would be many) who have an interest in the problems of their less fortunate fellows and in the machinery used by society to inflict punishment on offenders, and, inadvertently, their families.

D. C. Marsh

Probability and Statistics

By H. Freudenthal. Pp. v+139. (Amsterdam, London and New York: Elsevier Publishing Co., 1965.) 30*s*.

A SCIENTIST or technologist with modest mathematical skill and no fear of unfamiliar notation could gain considerable insight into statistical thought from this book. He would not advance far in learning standard statistical techniques for use in his own problems because the book does not attempt their systematic presentation. Prof. Freudenthal's strength lies in a largely intuitive approach allied to an attractively easy style. He does not retreat from difficulties, but he refuses to conceal the essential features of a proof by trappings of mathematical rigour appropriate only to more advanced texts.

Early chapters are largely concerned with probability theory, made vivid by excellent simple examples of independence and conditionality. The implication that sampling with replacement is theoretically preferable to sampling without replacement is strange, but possibly translation from the Dutch has permitted entry of a confusion between simplicity of theory and practical desirability. Prof. Freudenthal uses the central limit theorem to justify widespread practical dependence on the normal distribution. The final one-third of the book has chapters concerned with sampling inspection (including sequential sampling), distribution-free tests, games and strategy, and population genetics. None of these topics is taken far, and all are regarded a little abstractly as playgrounds for mathematicians rather than as fields of investigation in which the statistician must make a great contribution. In the context of a book that may make an intellectual appeal to mature readers but that is not suitable as an introductory text, they play their part well. The book can be confidently recommended to those who find its form seeming to meet their needs, but disregarded as of no fundamental importance by those to whom its somewhat unusual style makes no appeal. Of few books on probability and statistics can both these statements be made!

D. J. Finney

ALGOL 60 Implementation

The Translation and Use of ALGOL 60 Programs on a Computer. By B. Randell and L. J. Russell. (A.P.I.C. Studies in Data Processing, No. 5.) Pp. xiv+418. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 84*s*.

THE introduction of automatic computer languages has opened up the use of computers to a large public by making the programming of digital computers much easier. This is due to the development of compilers: the large programmes which translate automatic languages, which are relatively easy to use, into machine languages which are much more difficult to use. Initially automatic languages and their compilers were very much empirical *ad hoc* affairs; the writing of compilers was more of a craft than a science. But recently this state of affairs has changed, an important step in this direction being the publication some five years ago of the ALGOL report. Using the notations and techniques of mathematical logic, this defines precisely a programming language suitable for scientific computations. This stimulated the systematic study of compiling methods and many papers on the subject began to appear.

This book is an excellent and welcome contribution to the literature; it will make more accessible to a large public some of the more commonly used and basic compiler techniques. The compiler described produces in one pass an object programme which is obeyed interpretively by a control routine during run time. This may not be very efficient, but many of the concepts explained in this book are relevant to other forms of compilers. In particular, repeated use is made of the concept of stack or push-down store.

Because this is among the first books on the subject, the form and contents of the book may put off the uninitiated reader, but it is well worth persevering with. An attractive aspect of the book is the large number of very short programmes illustrating the many difficulties a compiler has to deal with.

I. M. Khabaza

Einführung in die Atomphysik

Von Dr. Wolfgang Finkelnburg. Neunte und Zehnte Ergänzte und Neubearbeitete Auflage. Pp. xii+552. (Berlin, Göttingen und Heidelberg: Springer-Verlag, 1964.) 45 D.M.

THE present issue of *Einführung in die Atomphysik*, by Prof. W. Finkelnburg, is the nineteenth and twentieth reprint, revised and brought up to date by the author, who is a member of the Siemens–Schuckertwerke group in Erlangen. It is intended for physicists in the applied fields, engineers, chemists and technical personnel. Its scope is very wide, comprising, besides atomic physics, extensive chapters on quantum mechanics, nuclear physics, 'elementary' particles, molecular and solid-state physics. The author is manifestly an experienced pedagogue who leads the reader in carefully planned steps through the various phases of an argument. It is a magnificent and, on the whole, successful effort to provide a readable text of a complex scientific field.

It is, however, clear that the maximum one can achieve in the circumstances is plausibility, which is probably what the author aimed at. The question then arises if it is a good plan to incorporate a fair proportion of the wave mechanical formalism (according to Sommerfeld), without the mathematical background (see p. 360, where the formula is completely without meaning). It must be admitted that the figures of electron distribution and some wave functions are excellent.

The book, as such, comes up to the expectation one holds for a production from the publishing firm of Springer; it is quite cheap at 45 D.M., and will no doubt greatly contribute to the education of that most important part of the scientific community who apply science.

E. Bretscher

Physique des Semiconducteurs

Comptes Rendus du 7e Congrès International. (Physics of Semiconductors: Proceedings of the 7th International Conference.) Pp. xxii+1368. (Paris: Dunod, 1964.) 145 francs.

PHYSIQUE des Semiconducteurs: Comptes Rendus du 7e Congrès International contains about 200 distinct original items, five of which are reports on symposia on related topics. It will be an invaluable work of reference pointing to other and fuller papers on certain topics, and indicating the major aspects of present interest in the field. It has been remarked that this field, like women, has become more sophisticated with age, and this volume certainly gives evidence in favour of this assertion. More accurate measurements, and more detailed calculations are reported here, of quantities the broad significance of which has in many cases been known for some years. This impression is strengthened by the fact that the details of the symposia on radiative recombination, radiation damage, and solid-state plasma effects, all very active fields, have been published separately. This still leaves interesting effects within the scope of this volume—effects which were not expected two years before (during the corresponding meeting at Exeter in 1962). The relativistic correction to band structure calculations is an example. The importance of lasers is another example, for they have enhanced the interest in studies of electron transitions in solids, which may compete with radiative transitions. Papers in this volume deal with these and other important effects. It is clear that this field is con-

tinuing to throw up important new developments, and it is reasonable to expect an average of one important development per annum to be maintained until the next volume of this series (reporting the meeting in Tokyo in 1966) is reviewed in these columns.

P. T. Landsberg

Counterexamples in Analysis

By Prof. Bernard R. Gelbaum and Prof. John M. H. Olmsted. (The Mathesis Series.) Pp. xxiv+194. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1964.) 7.95 dollars.

THE schoolboy learns that the harmonic series does not converge, although its nth term tends to zero; the undergraduate is shown Weierstrass's function, which is continuous but not differentiable; at a later stage, a mathematician's pleasing speculation may be disrupted by an instance of its falsity. The construction of counter-examples is a not unimportant part of the technique of the analyst, as, for example, in the theory of Fourier series, and this collection of some 250 items may serve for profit as well as for amusement. The authors have made a selection from number systems, functions, limits, differentiability, integration, convergence, set and measure theory in one and two dimensions, metric, topological and function spaces. Almost any dip will come up with something pleasing; a random opening provided functions f and g such that f^2 and g^2 are integrable but $(f+g)^2$ is not. But the book will be more seriously useful to the teacher of analysis, who is often in need of examples of this type to show why the conditions imposed on a theorem are necessary, and to the young research worker who may have to test heuristic ideas by hard fact.

T. A. A. Broadbent

Radioactive Fallout, Soils, Plants, Foods, Man

Edited by E. B. Fowler. Pp. 317. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 80*s*.

THE evaluation of the significance of dietary contamination with radioactive fall-out requires the co-operation of many disciplines, and our understanding of this subject has owed much to meetings at which meteorologists, health physicists, agricultural scientists and medical radiobiologists have brought together the relevant information from their various subjects.

Two particularly important meetings of this type were arranged in 1959 by the University of Minnesota and the Food and Agriculture Organization in Rome. The publications to which they led in the following year largely paved the way to the first authoritative and comprehensive assessment of mechanisms of dietary contamination which the United Nations Scientific Committee on the Effects of Atomic Radiation included in its 1962 report. Other meetings with the same general objective had meanwhile been held, among them being a symposium of the American Chemical Society, at Cleveland, Ohio, in April 1960.

The volume under review is based on the papers presented at the Ohio meeting. The editor's preface shows his consciousness of the disadvantage of delay in publishing reports of symposia on rapidly developing subjects, and an endeavour was made to revise and to 'up-date' contributions. This was notably achieved in the discussion of the "Transfer of Fallout Radionuclides from Diet to Man", by Drs. Wasserman Lengemann, Thompson and Comar, in a chapter which contains about half the references to publications dated 1962 or later which are to be found in the entire volume. Read in 1965, some of the other chapters are mainly of historical interest. Not only are a number of transfer routes of much smaller importance than was sometimes thought in 1960 but also interest in the significance of fall-out now centres primarily on the effects of weapon tests in 1961 and 1962 which were of considerably greater magnitude than the earlier series.

Piante Medicinali

Chimica Farmacologia e Terapia, Vol. 2. Da R. Benigni, C. Capra and P. E. Cattorini. Pp. 731–1832. (Milan: Inverni e della Beffa, 1964.) L.12,000.

THIS is the second part of a comprehensive Italian work on medicinal plants, Volume 1 having already been reviewed in these pages (*Nature*, 196, 609; 1962). Plants are arranged according to their Italian common name. Volume 1 covered the letters *A–H*. The present volume deals with the rest of the alphabet, representing some 120 different species. As in the case of the earlier volume, there are notes on etymology, nomenclature, habitat, parts used, active principle, therapeutical properties, pharmacology, and chemical composition. There is special emphasis on the chemistry of the plants, which is covered in some detail and the latest information incorporated. This applies particularly to plants that have recently attracted special attention, for example, *Rauwolfia serpentina* has 149 pages devoted to it and there are more than 800 references. *Strophanthus* (*Strophanthus kombé*) covers 25 pages, with 161 references.

Other well-known medicinal plants dealt with in this volume include golden seal, Iceland moss, ipecacuanha, jaborandi, liquorice, lobelia, olive, opium poppy, peppermint, rhubarb, senna and strychnine or nux-vomica. Many of the other species treated, although well known, are of little importance medicinally to-day. Nevertheless it is of value to have so much information in relation to them brought together. The common horse-chestnut (*Aesculus hippocastanum*) is not usually looked on as a medicinal plant, yet 14 pages are devoted to it along with 38 references. This may be because of the saponin it contains.

F. N. Howes

Pollen Physiology and Fertilization

Edited by H. F. Linskens. (A Symposium held at the University of Nijmegen, The Netherlands, August 1963.) Pp. xii+257. (Amsterdam: North-Holland Publishing Company, 1964.) 80*s*.

IN August 1963 a symposium was organized at the University of Nijmegen to bring together a number of people interested in the study of pollen tube formation and germination, and the processes leading up to the formation of the zygote in higher plants. The papers and discussions given at this symposium are now published under the title *Pollen Physiology and Fertilization*, edited by Prof. H. F. Linskens.

The symposium was organized in seven sections each of about three or four papers followed by a tape-recorded discussion, Subjects included the physiology of the embryo sac, and the biochemistry of pollen wall formation; pollen tube metabolism; the effects of boron on growth; chemotropism and sections on controlled fertilization and incompatibility. Particular interest is derived from the high proportion of papers in which relatively modern techniques such as electron microscopy, histochemistry and *in vivo* methods of controlling fertilization are used to investigate the problems raised.

The publication of papers read at any specialized symposium inevitably results in the reappearance of a fairly high proportion of material already familiar as a result of its appearance in regular journals, and this is no exception. Nevertheless, this volume does provide a readily accessible collection in one place of contributions by a number of well-known authorities on the subject; and the convenience for comparison and survey which this provides, combined with the general high standard, or controversial interest, of the papers will make it a welcome addition to the collection of anyone interested in the physiology of pollen germination and fertilization.

P. A. Thompson

ORIGIN AND SIGNIFICANCE OF THE POWER OF VOCAL IMITATION: WITH SPECIAL REFERENCE TO THE ANTIPHONAL SINGING OF BIRDS

By Dr. W. H. THORPE, F.R.S.
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AND
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THERE are few abilities which separate man more clearly from other members of the animal kingdom than his powers of imitation. Even among the primates the ability to learn a new action as a result of having seen it performed by another member of the species has only been established relatively recently; and convincing examples among mammals below the primate level are sparse indeed[1].

When we come to consider vocal imitation the difference between man and the other mammals is even more striking. Even with the chimpanzee a capacity for vocal imitation seems to be almost non-existent and no satisfactory scientific evidence seems yet to have been presented on behalf of claims made for the powers of vocal imitation among porpoises[2,3]. This remarkable difference is considered by Konorski[4], as a result of his analysis of various forms of speech disorder, as comparable with the audio-verbal aphasia which results from certain types of brain lesion in man. Lesions which have this effect are shown to have damaged either the postero-superior part of the temporal lobe, or to have severed the 'arcuate bundle' which originates in this area and runs to the lateral frontal regions. According to Konorski, it is a striking fact that there is nothing exactly comparable with this 'arcuate bundle' known in non-human mammals, and it is this absence which is thought to account for the lack of powers of vocal imitation.

When we come to compare the imitative abilities of the birds with those of the mammals, we find that while the ability of the former group to imitate bodily actions is probably even less than it is in mammals, the powers of vocal imitations are of an astonishingly high order, being even comparable in some cases with those possessed by man. The origin and significance of this difference raise, therefore, most puzzling problems. Unfortunately, on the neurological side, nothing can be said; for the investigation of the bird's central nervous system has not yet nearly reached the stage at which we can say whether or not there is any structure functionally comparable to the 'arcuate bundle' of the human brain. The only birds on which modern neurological investigations are being actively carried out are the doves; but it appears[5] that these are incapable of imitation. We have, therefore, to come down to behavioural evidence, as this does indeed provide some interesting clues.

In the Oscines, or song birds, at least those of the temperate regions of the world, the most usual function of the full-song is to serve as a territorial proclamation by the male establishing claim to a territory and, as a substitute for fighting, defending it. Thus it serves as a signal which is sufficiently stereotyped to function as a recognition mark characteristic of the species. But it is well known (refs. in Thorpe[6]) that in many species the song is capable of sufficient individual variation within the overall specific fixity of pattern to differentiate one individual bird from another. Thus, the song comes to serve two functions—specific recognition and individual recognition.

This individual differentiation of the song is often developed by imitative learning, the bird in its first year either imitating its parents' song or, perhaps more often, learning individual differences from other members of its species when it first comes to sing in competition with them during the process of establishing a territory during its first breeding season. Thus, chaffinches (*Fringilla coelebs*) reared in isolation produce an abnormally simple song which is mainly accounted for by the hereditary make-up. Only when the bird is reared in contact with its own kind does it develop the full species pattern and add the aforementioned individual characteristics. Thus, experience influences the selectivity for the song which will be imitated, and in early spring the bird tailors its own song to match the patterns which it has heard. There is, therefore, a functional explanation for the ability to imitate the vocalizations of one's own species even where the prime function of the song is territorial advertisement and defence.

But, as everyone knows, the imitative performance of a number of birds goes far beyond this. There are species, such as the starling (*Sturnus vulgaris*), the mocking bird (*Mimus polyglottos*) and the marshwarbler (*Acrocephalus palustris*), which include notes and phrases from many other species in their own song. Even more remarkable are the 'true' talking birds, especially the members of the parrot family and the grackle, known as the 'Indian mynah' (*Gracula religiosa*), which can imitate not only human speech to astounding perfection but also a seemingly endless variety of other noises. This extreme imitative ability of certain groups poses a real evolutionary problem, since it appears not to be used at all except under conditions of domestication. In other words, it seems as if the bird is in some way 'pre-adapted' for the performance of dazzling feats of imitative virtuosity which conditions of life in the wild never require of it.

In the tropics, although there are many species of birds the song of which is doubtless just as territorial in function as is usual in the temperate regions, the ornithologist is also struck by the number of examples where song appears much less aggressive in intent and where its function is apparently as a social signal, for maintaining pair and family bonds and as part of the sexual display, rather than a territorial one. Moreover, it is perhaps significant that most of the outstanding vocal imitators are found among tropical or sub-tropical species. It is suggested that the extreme developments of the imitative ability occur where the main function of the song is to provide for social recognition and cohesion rather than for territorial defence. This seems to apply specially in the tropics.

In 1903 Waite[7] described how two captive Australian magpies (*Gymnorhina tibican*) learned to sing a fifteen-note melody, played to them on the flute, as an antiphonal duet. When the younger bird died the survivor resumed the performance of the whole; which it had never been heard to produce during the years when it had a companion. A rather similar case of the antiphonal division of a learned melody between a pet canary (*Serinus c. canarius*) and a captive bullfinch (*Pyrrhula pyrrhula*) was described in the same year by the famous musician Sir George Henschel[8]. Recently, Gwinner and Kneutgen[9] described how, with two pairs of captive ravens (*Corvus corax*) and three pairs of the shama (*Copsychus mala-*

baricus), the males and females each had sounds or song elements which were principally, if not exclusively, their own private utterances and were not normally used by their mates. However, when the partners were absent, the remaining bird would use the sounds normally reserved for his partner, with the result that the said partner would return as quickly as possible as if called by name. (We have both experimental and field evidence for the same effect with *Laniarius aethiopicus major*.) Gwinner and Kneutgen find the method to be particularly effective if it is the male who is absent, since, they suggest, there is nothing more stimulating to a mated male than to hear its own repertoire repeated in its own territory. Thus, the vocalizations of these species appear to be used for securing the return of the desired partner. It seems from the work of Zur Strassen[10] that, contrary to much previous supposition, parrots can also use their learnt vocalizations in a 'purposive' manner for securing food or other particular needs.

The study of the antiphonal singing of certain tropical birds, on which we have now been engaged for some years, is particularly relevant to these problems. Our investigations have been concerned with three widely separated groups of birds, one non-passerine and two passerine, namely, the barbets, *Trachyphonus* spp. (Capitonidae), fantail warblers of the genus *Cisticola* (Muscicapidae, sub-family Sylviinae) and shrikes of the genus *Laniarius* (Laniidae including Laniariidae). In all these groups one encounters species capable of a duetting in which the notes of the male and female are different—the sexes alternating to sing antiphonally but nevertheless with a very precise and exactly maintained time interval between each contribution. One of us[11] has already published an account of the antiphonal singing of *Laniarius erythrogaster*, where the response time with which the female answers the male is of the order of 150 msec or less, with a standard deviation over a long series of 12·6 msec. Here it was suggested that it is the exact precision of timing of the answer of the second bird which is the most likely basis for the mutual recognition in dense foliage rather than any individual difference in tonal quality or structure of the notes—although this is not ruled out.

Since this work was published, Grimes[13] has obtained almost identical results with another species in West Africa—*Laniarius barbarus*.

However, from the point of view of the general problem already outlined here, it is the species that we are now mainly concerned with, namely, *Laniarius aethiopicus*—the tropical bou-bou shrike—which is providing most of the important results to date. Although a great deal remains to be done, a number of essential points already seem clear, and it is the object of the present article to outline the most relevant of them. In this species both male and female have a considerable vocabulary which, it seems, they utilize so as to work out with practice a series of duet patterns by which, presumably, when the birds are no longer in sight of each other mutual recognition is still possible. This work is proceeding in the field in Kenya and Uganda, and in tropical aviaries at the Cambridge Sub-Department of Animal Behaviour. The following essential points seem to be clear:

(1) Although there may be periods of solitary 'practice' during the juvenile stage, the duet is worked out during

Fig. 1. *Laniarius aethiopicus sublacteus*. Vipingo, Kilifi, Kenya. December, 1954. *N.B.* All the illustrations are given at approximately scientific pitch (middle '*C*' = 256 c/s). Unless otherwise stated, as here, all the figures refer to race *major*

Fig. 2. *L. aethiopicus*. Dundori, Nakuru, Kenya, March 17, 1964

Fig. 3. *L. aethiopicus*. Kabale, Uganda, February 15, 1962

Fig. 4. *L. aethiopicus*. Meadow Point, Lake Nakuru, Kenya, March 17, 1964

Fig. 5. *L. aethiopicus*. Hippo Pool, Lake Nakuru, Kenya, March 17, 1964. Note that this is a rather more elaborate duet than the previous ones. The contribution of the two birds is not indicated in this case since it seemed to vary a good deal

Fig. 6. *L. aethiopicus mossambicus*. San Martino, Mozambique Coast (C. Haagner). The timing in this example is very precise, but the bar length might vary between 0·75 and 1·5 sec

Fig. 7. *L. aethiopicus mossambicus*. San Martino, Mozambique Coast (C. Haagner). This is a duet with a more complex time pattern. Bar length 1·5 sec

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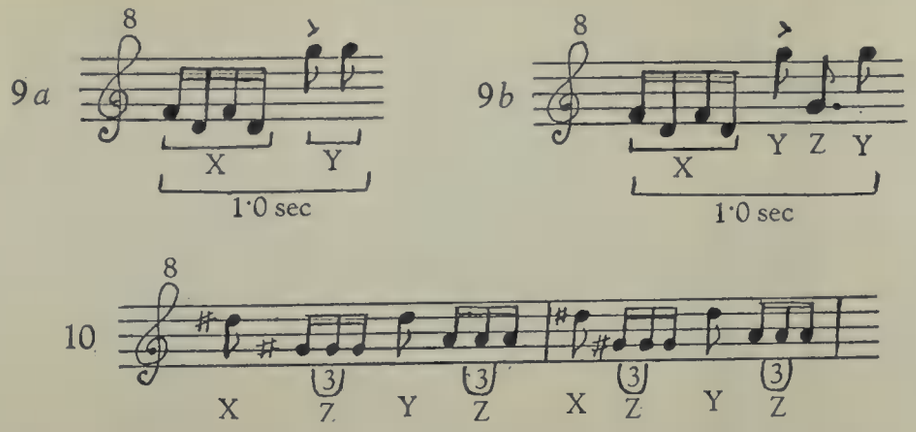

Fig. 9. *a* and *b*. *L. aethiopicus*. Lake Bunyoni, Kabale, Uganda, February 14, 1962. Fig. 9*a* represents a duet pattern heard as a very long, precisely timed series. During one considerable stretch of this series a third bird 'Z' joined in. It was far away from the others, but nevertheless inserted its single note remarkably accurately (Fig 9*b*). It tended to intervene in every second duet of 'X' and 'Y'

Fig. 10. *L. aethiopicus*. Dundori, Nakuru, Kenya, April 3, 1964. A remarkable trio. All three birds were in the same tree. Note that bird 'X' gave ■ *D* sharp every two seconds and bird 'Y' a *D* sharp every other two seconds while bird 'Z' gave ■ *G* sharp and an *A* natural every other two seconds in alternation

with 'Y'. To date, rather more than 135 different duet patterns of *Laniarius aethiopicus major* have been recorded and analysed. A characteristic selection of these is here shown. Some races of *Laniarius aethiopicus* (for example, race *major*) appear to produce a greater variety of duet pattern and to be more flexible in their combinations; but this may merely be due to the fact that this is the race which it has so far been possible to examine most intensively. So far we have encountered one pair of birds in Nairobi which produced twelve different duet combinations, and another pair at Nakuru, Kenya, which produced seventeen duet patterns in the course of a single day (Fig. 8).

Figs. 1–7 show a characteristic selection of duet patterns, proceeding from the rather simple to the more complex. Note also the way in which the total time taken by a duet can be varied considerably even by a single pair of birds more or less irrespective of the number of notes which it contains. Note, too, how the second bird may add or interpolate a single note within an already well-established pattern. Sometimes this note almost exactly duplicates one of the existing notes, in which case it can usually be distinguished only when the record is analysed by sound spectrograph. At other times it can be distinctly heard—as in Fig. 8*k*.

The 'musicality' of these songs strikes all who hear them. This is partly because of the purity of tone and the low pitch as compared with the songs of most birds. But in addition to this we find the birds using a familiar musical tonal system in a strikingly 'orthodox' manner. The implications of this are now being investigated.

With several of these birds which sing antiphonally, trio singing is sometimes observed. The meaning of this is at present quite obscure. If often recalls the well-known visiting of fulmar petrel (*Fulmarus glacialis*), the 'piping parties' of the oyster-catcher (*Haematopus ostralegus*) and the dance of the spur-winged lapwing (*Belonopterus chilensis cayennensis*) described by W. H. Hudson[14]. In more than one instance of trio singing in *L. aethiopicus* observed by us, besides the three birds partaking, a fourth bird was observed standing silently by; possibly the mate of No. 3. Figs. 9 and 10 show examples of such trio singing in *L. aethiopicus major*. Trio singing is also very frequent in *Cossypha heuglini*, Heuglin's robin chat, which is also being studied in field and in aviary. An account of this will be reserved for later publication.

It is concluded that our observations so far give strong support to the suggestion put forward by Gwinner and Kneutgen, and also by Konrad Lorenz, that one of the major functions of the imitative ability of birds is to establish and strengthen the individual pair bond. We suggest that this will be so particularly in those species where territorial aggressiveness is not very marked—as is so often the case in the tropics. In *Laniarius aethiopicus* this ability enables each bird to learn the normal contribution of its mate as well as its own. It can thus use this appropriately for maintaining contact with the partner and recalling him or her when absent. If this is so, then the extreme of apparently unused imitative ability of birds such as parrots and mynahs received a plausible explanation. It may well be that in the wild these birds use their powers for imitating the minute idiosyncrasies and inflexions of their partners or other members of the group. If this proved to be true, it is no longer necessary to suppose a mysterious 'pre-adaptation'.

Many, perhaps most, mammals recognize their associates primarily by smell and secondarily by sight. Voice and its recognition seem to come (with certain exceptions, among them possibly the Canidae and some Ungulates) a long way behind in importance. With birds, creatures of vision as they are and with the powers of olfaction greatly attenuated, audio-vocal recognition is probably paramount. But how did it come about that *Homo sapiens* so greatly outdistances his fellow Primates in his powers of using his auditory sense for social purposes?

We thank Mr. C. E. Cade, of Nairobi, for his painstaking work with hand-reared birds in the early stages of investigation. We also thank Mrs. Avril Royston, formerly of Sotik, now of Nakuru, Kenya, for her work in catching and hand-rearing birds—particularly shrikes. Without her expert help the aviary observations so essential to this work would have been impossible. We are also much indebted to Mr. Clem Haagner for allowing us to use some of his tape recordings of race *mossambicus*. Much of the cost of the work has been defrayed by grants from the Royal Society.

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THE INTERNATIONAL BIOLOGICAL PROGRAMME*

By Dr. E. B. WORTHINGTON
Scientific Director (Designate)

THE International Biological Programme is now well into its first year—the first of seven or eight. It is concerned with "the biological basis of productivity and human welfare". It is divided into two phases: Phase I, which consists mainly of design and feasibility studies, to occupy most of 1965 and 1966; Phase II, the operational programme, to commence in 1967 and carry on for about five years. Beyond these simple facts I doubt if there are many people who know much about the Programme, for we have been so busy planning it that little thought has been given to advertising it.

The International Biological Programme is a programme which, because of the effects of various factors, particularly the climate, must extend over a number of years, since it is obvious that an investigation of growth in a summer such as we have had this year would be quite different from a similar study undertaken last year. It has, however, drawn some of its inspiration from the International Geophysical Year. Indeed, if one looks at the development of the sciences during the past few decades one is struck by the high peaks of achievement reached in international collaboration and also through international competition in the physical sciences. Research on the ionosphere, the atmosphere, and the geosphere has been greatly stimulated by organized activities—the International Polar Years, the International Geophysical Year and the International Years of the Quiet Sun which have developed one from the other. Programmes are going forward strongly, too, in connexion with the hydrosphere, as arranged by the Intergovernmental Oceanographic Commission and the International Hydrological Decade. But in such investigations the biosphere has been almost completely neglected. Yet on the natural resources of this Earth—especially the renewable ones which come from biological productivity—the future of mankind depends. They are much more important to most human beings than getting to the Moon or taking close-up photographs of Mars. One could even argue that it is more important to humankind to ensure a sustained yield of fish from the North Sea than to discover and extract the oil that may lie beneath it.

What do we mean by biology within the context of the International Biological Programme? To some, biology means the life sciences as opposed to the earth sciences; to some it stimulates thoughts of the inner workings of the cell; and to some perhaps, merely the facts of human reproduction. For the purposes of an International Biological Programme, I think the best definition is the biosphere, that is the living layer around the Earth, on land, in the waters and in the air.

This word biosphere, by the way, seems to present a problem in semantics. I have heard it explained by a television astronomer as a concentric region of the solar system, comprising the orbits of Venus, the Earth and Mars, where there is some reason to believe that life as we know it exists. I use it, however, as the layer which supports life in, on and around the Earth, for the International Biological Programme is likely to have little time or opportunity to extend its activities to other planets.

Some biologists are inclined to think that the International Biological Programme is rather a waste of effort and money. They argue that opportunities for the exchange of knowledge and ideas are not lacking, but are almost overburdening, for international unions and associations exist in every branch of biology. From spring to autumn scarcely a week passes without some international biological congress, often several running contemporaneously, even in the same country. Is this not enough?

The counter argument is that we scientists concerned with environmental biology have to take but one look at our colleagues concerned with the physical environment to appreciate what a degree of organization can do. Few would deny that studies of this kind of biology, concerned with the organism in relation to its environment, which had such a splendid start in the past century, have been sadly overtaken in this century by studies in the physical sciences. In the long run, I believe that the International Biological Programme will be looked on as a major effort—and a successful one—to redress this balance. In doing so it will provide a part of the fundamental knowledge which to-day is so much needed so that biological science can be applied more effectively to the provision of human needs.

History

Before examining the content of the International Biological Programme we should consider how the idea came into being. British scientists have taken a prominent part in this. Sir Rudolph Peters some years ago, when president of the International Council of Scientific Unions (ICSU), took a major part in the conception of the International Biological Programme. Prof. H. W. Thompson of Oxford, the current president of ICSU, did a good job as midwife during the first International Biological Programme Assembly held in Paris last year. Prof. C. H. Waddington, as president of IUBS, has been a good doctor in the pre- and post-natal clinics; while the president, officers and secretariat of the Royal Society, by priming and holding the feeding-bottle, have enormously helped the infant's early growth.

How the ideas leading up to the International Biological Programme have fluctuated and developed has been related by Prof. Montalenti, the Italian geneticist who led much of the preparatory work. Initially, it was thought that the programme should concentrate on the human organism, and especially population dynamics and genetics. Later there was a body of opinion which thought it could involve itself best on the preservation and conservation of biological systems in different parts of the world, especially those which are most endangered by economic progress. Finally, these and other approaches have been brought together in such a way as to be of interest to a rather broad spectrum of biological scientists. The objective, as defined in 1964, is to ensure a world-wide study of: (*a*) organic production on the land, in fresh waters, and in the seas, and the potentialities and uses of new as well as of existing natural resources; (*b*) human adaptability to changing conditions. The programme should not range through the entire field of biology but should be limited to basic biological studies, related to productivity and human welfare, which will benefit from international collaboration, and are urgent because of the rapid rate of the changes taking place in all environments throughout the world.

Thus, of two major problems now facing the human species on Earth—of controlling his rate of reproduction

* Substance of an Evening Discourse delivered on September 3 at the meeting in Cambridge of the British Association for the Advancement of Science.

and of increasing the biological product on which he subsists—the International Biological Programme is concerned with the second. The advances in techniques to control reproduction which have been produced in the past decade have reached a point at which there is more need for sociologists to apply them than for biologists to invent new ones. Yet the attributes and potentialities of man are by no means left out of the programme, as I will explain.

Principles

The programme is based on a number of principles. First, it is a directional and a selected programme. It is not a free-for-all, nor is all research bearing on productivity and human adaptability automatically incorporated into it. The initials IBP can be regarded, if you like, as a kind of status symbol, and to ensure that this is not misused we have a sifting arrangement for projects at the national level and later at international level before admission into the programme. The tag of IBP does not, of course, mean that the project is necessarily good science, nor that other work on biological productivity conducted outside IBP is not good science. It merely implies that those immediately concerned with IBP agree that the project fits in and is likely to advance materially the objects of the programme.

Secondly, the programme is concerned throughout with the fundamental approach to research. It starts from the premise that the applied sciences of biological production, such as agriculture, forestry, fisheries, have in recent decades gone striding ahead more quickly than the fundamental understanding of the causes of productivity. Similarly, the sciences of man, medicine and some of the applied social sciences, have in some ways outpaced fundamental understanding of mankind and the differences between one man and another. The International Biological Programme is an opportunity to restore the balance and to provide new organized knowledge as a springboard for the technologies to take further plunges into progress.

Thirdly, the International Biological Programme is urgent. This is not only because of the steady growth of the pressure of human populations on renewable resources, but also because many of the situations in the world, both biological and human, are changing so rapidly that they will soon cease to exist. The International Biological Programme is not a preservationist body, for our approach is dynamic not static, conservation rather than preservation; but the perpetuation of sample biological systems for future biologists to study is definitely among its objectives.

Fourthly, and this could perhaps almost go without saying, the International Biological Programme is limited to research which could benefit from international co-operation. There are some scientists, and great ones at that, who do not readily co-operate with others and whose special attributes lead them to penetrate deeply on a narrow front the unknown, behind locked doors so to speak, rather than, by a sharing of knowledge, to advance with others along a broad one. The International Biological Programme is clearly not designed for the isolationist researcher, although his findings might well be picked up and developed as a Programme project.

Divisions of the Programme

In order to get to grips with what inevitably is a very large content, the International Biological Programme is divided into seven sections. One of the first and most obvious divisions was into the biological communities of the land, of fresh waters, and of the sea, because terrestrial ecology, limnology, and oceanography have each developed their own discipline of research, although biological energy often flows from one to another. These three sections are known by their initials; PT for Productivity of Terrestrial Communities; PF for Productivity of Freshwater Communities; PM for Productivity of Marine Communities.

All three have their problems of conservation, including the definition, description and management of prescribed areas or samples of living communities. The problems of conservation on the land are more extensive and generally more complex than those of the waters. They include such concepts as national parks, nature reserves, and sites of special scientific interest. Therefore, there is a special section of IBP labelled CT for Conservation of Terrestrial Communities. Each of the three, moreover, presents different facets of the processes of production—photosynthesis (utilization of solar energy) and the nitrogen cycle—this section is called Production Processes (PP). Each of the three may reveal new resources of value to mankind or new methods applicable to the use and conservation of resources, which require fundamental study; this will be carried out within the section named UM for Use and Management of biological resources. Finally, the human problems, which bring in another series of disciplines, notably physiology and anthropology, will be studied within the Section of HA, Human Adaptability. This makes seven: PT, PP, CT, PF, PM, HA and UM.

Each of these sections is headed by an international convener and sectional committee, and is busy in producing a five-year programme of its own. Two of the conveners are British—Mr. Max Nicholson of CT and Prof. J. S. Weiner of HA. The seven sections are, of course, closely related to each other, but also to other programmes going on during the same period, for example PF to the International Hydrological Decade.

IBP consists of the sum of its parts, not only in the sense of containing seven different programmes, but also in the sense that the bulk of the research will be undertaken and financed nationally, not internationally. Many countries are at present busy preparing their national programmes, and of these one of the most advanced is that for the United Kingdom, now published by the Royal Society.

Examples of Research

Let us take a few examples of the kind of work which IBP will contain, and think especially of the British contribution. Although a great deal has yet to be learned about our own biological communities and our own people, some of the thinking has been on the lines that we should use the knowledge and abilities of a good many scientists overseas, where ignorance about biological productivity is still very great. Thus the U.K. programme in its PT section aims to establish two bases for IBP work in the warmer regions overseas, in co-operation, of course, with the countries concerned. One of these is likely to be in savannah country, at the Cambridge Nuffield Unit of Tropical Animal Ecology, situated in the Queen Elizabeth Park in Uganda; another at a site yet to be determined in an area of tropical rain forest. At these bases, in co-operation with local scientists, it is hoped to undertake investigations of productivity in depth—primary production of the trees, shrubs, grasses and herbs, secondary by herbivorous mammals and insects, tertiary by predators and parasites.

It is hoped to quantify as well as qualify each link in the food chain so as to obtain data to compare production in the wild with production from the tame, for example, where domestic animals and plants have been established on land used for agriculture.

Passing to another section, namely CT, it is pleasing to report that something has been actually achieved. In the spring of 1964 and again in 1965, expeditions went from Great Britain to the desert lands of Jordan, and made a survey especially of the Azraq Oasis and its environs, to the east of Amman. This has resulted in several things: a useful IBP booklet entitled *An*

Approach to the Rapid Description and Mapping of Biological Habitats, and more important, in detailed recommendations about the creation of national parks in Jordan. Further preparatory work has been based on the Azraq Oasis where the park headquarters and warden are now established with American aid. Within a week or two, two experienced members of the Nature Conservancy's staff are going there to prepare a management plan, applying the experience in management of wild areas gained in this country. Meanwhile, in August H.M. The King of Jordan publicly announced that he will set up the Azraq National Park, and also that Jordan will participate in the IBP by establishing at the Park's centre an institute for biological and human investigations of desert and oasis conditions. This indeed will make a useful counterpart to the proposed IBP bases in savannah and rain forest to which I have already referred.

From the PF section I will draw two examples of British initiative: one is an intensive study of the biological productivity of a reach of the Thames, organized from the University of Reading. This is dealing with primary and secondary production and will include studies on the feeding and population dynamics of a considerable number of fish species. It will fill a major gap in our knowledge of British fresh waters, for most intensive work has been devoted to lakes and very little to rivers. The other example relates to research in tropical lakes. In terms of food supplies through fisheries, and water supplies for irrigated agriculture, they are of much greater importance to the world than the little lakes in our own country. So the programme includes the establishment, based on Britain, of a team of limnologists for work in the tropics, especially Africa. This takes advantage of the fact that we happen to have in Britain a rather particular expertise in tropical lakes.

During the present planning phase each of the seven sections has already held, or has arranged, a number of symposia consisting of selected specialists from all over the world to discuss methods of research in particular spheres and to prepare handbooks. The object here is not to 'straight-jacket' the methods in use in IBP, for field biology is at present advancing very rapidly through the development of old methods and the devising of new ones; but it is to agree on those proved methods which can be advised for general use throughout the world and can be relied on to produce comparable results. A good example was a symposium held in Aberdeen and Cambridge in September concerning research on large herbivorous mammals. In Australia, studies of kangaroos and introduced sheep suggest that the former may be more effective converters of grass into meat than the latter; if only kangaroos produced wool instead of hair, they might be the basis of Australian prosperity. In Africa there are indications that the broad spectrum of indigenous African mammals, such as giraffe, antelopes, buffalo and wart hog, may be better agents of secondary production of meat than the exotic cattle, sheep and goats which have replaced them in many areas and are apt to depress the yield of the habitat. In Scotland we have the Island of Rhum, where management of red deer by the Nature Conservancy has resulted in more venison coming off the island than mutton under the former agricultural management and at the same time it seems as though the level of primary production of the vegetation has been raised. This symposium brought experiences of this sort together and advised on methods for future work under IBP from the three points of view—the ecological, the physiological and the pathological.

Another example I want to give is one with which I am personally involved just at present, namely research on large man-made lakes. The basic reason for creating these is generally for hydroelectric power: for example, at Kariba, Volta and Kainji in Africa, Brokopondo in Surinam, and a number of large impoundments in North America. Sometimes water conservation for irrigation is equally or more important, as in the Aswan High Dam in Egypt. But the influence of these great sheets of water will extend not only to producing power and water but also to many other human needs, and has a profound bearing on the biology of large regions. Anyone familiar with Africa, for example, will realize the fundamental influence of the great natural lakes on climate, vegetation, fisheries, water supply for man and stock, transport, sites for urban development, and the rest. Since the period of IBP happens to coincide with the period of constructing several new great impoundments and of the biological changes consequent on several of those already completed, their study will take quite a significant place in the programme. Here again, it is good to note that Britain has taken an initiative, and as I speak a group of eight scientists drawn from Liverpool and other universities in Britain and Nigeria, and supported financially by the Ministry of Overseas Development, are undertaking a preliminary investigation of the part of the River Niger where Mungo Park met his end in 1806, the reaches which, in a year or more, will form a five-hundred square mile lake impounded by the dam at Kainji. The group has now been joined by several American and African sociologists who are extending the aquatic study to the 50,000 or so people now living below the future water-level. Under the leadership of local people, assisted by scientists from outside, and with funds which we hope will be provided by the special fund of the United Nations, it is hoped to establish a co-ordinated programme of study on these great impoundments.

I have been speaking mainly about the purely biological aspects of IBP, but the section on human adaptability has so far been quite one of the most active. It brings in a lot of scientists in the spheres of medicine and of physical anthropology, and its programme is designed rather differently from some of the other sections. It is wide and comprehensive, divided into a large number of headings and subheadings. Research topics include environmental physiology, including tolerance of different human groups to cold, heat, and high altitude; fitness, growth and physique, which, incidentally, will include a survey of highly fit athletes; the genetics of populations, with international data centres to be developed on existing national ones such as the blood-group centre of the Medical Research Council in London; and lastly health, nutritional and epidemiological topics. Clearly a lot of this work will need to be done by groups of research workers travelling with their equipment to study communities in their natural environments. This involves mounting a good many quite complex expeditions to remote places. British workers under IBP/HA expect, for example, to work this autumn on the Hazda tribe around Lake Eyassi in Tanzania and the people of Bhutan in the very high Himalayas.

Organization

There is a school of scientific thought that resents all forms of direction or organization. Science is free: give us the funds and let us go unfettered in any direction where research leads, and we will show results. Good scientists should not become administrators; any who do so are so much loss to science. This is an attitude which some of us associate with the older universities, but it is quite widespread, within Government scientific service as well as outside it. I met it strongly expressed when discussing IBP recently in Uganda. Now this libertine approach to science does not face the realities of how the funds and facilities for research are to be produced, how the results are to be discussed and published, or how those results which have an element of application in them might be turned to practical use. Nevertheless, it is an attitude with which I personally, and many others who are concerned with IBP, have a good deal of sympathy. We have been influenced by it in thinking up the organization necessary for IBP, at least to the extent that we have

devised this as lightly as possible. We are trying to build a simple structure from the foundations and to make the roof no heavier than is necessary to protect those inside from the weather.

Starting from the top there is ICSU which has set up the Special Committee for the International Biological Programme (SCIBP), comparable with its scientific committees for Oceanic Research (SCOR) and Antarctic Research (SCAR). SCIBP is a democratic organization which holds a general assembly about every two years. It has a Swiss president, Prof. Jean Baer, vice-presidents from Italy, Poland, the United States and Great Britain, and a number of other members, some representing the main international unions in the biological sciences and some drawn from the different bio-geographical regions of the world. It includes also the seven international conveners of the sections of IBP. Unesco, FAO, WHO, SCOR, and SCAR have representatives on it. Each of the conveners has also his sectional committee of specialist members drawn from all over the world.

The central office for IBP is at 7 Marylebone Road, Regent's Park, London, in accommodation generously provided and furnished by the Government and the Royal Society. Each of the seven international conveners has his own office, although only four of them as yet have whole-time staff.

Publications of IBP are so far very few: a journal called *IBP News* was started in October 1964, and so far three numbers have been published. We are also starting a series of handbooks, and the first of these, being a general guide to the activities of HA section, has just gone to press.

The central funds, for running the offices and holding meetings and for publications, come from national dues paid in by each participating country. These are on a rather modest scale and in fact are at present insufficient. I am glad to say they are augmented by grants and loans from ICSU and Unesco, and a particularly generous grant recently made by the U.S. National Academy of Sciences. It is hoped that other countries will follow this excellent example.

The finance I have spoken of is concerned only with the organizational framework and, by international standards, is quite small. The cost of the actual research undertaken under the auspices of IBP in all parts of the world will naturally be quite large if the programme is to be at all worth while. As yet it is impossible to say how much it may cost, because very few national programmes have yet been prepared in detail although a number are in active preparation. If we take the British programme as a model, and if some twenty major nations have comparable programmes and twenty or thirty others prepare smaller programmes, the total is bound to run to several million pounds. The finance for national programmes will of course be provided nationally: in this first year of design and feasibility studies, the British Treasury has provided some £80,000 specially for IBP; added to the normal votes for the Royal Society and the Nature Conservancy. This will enable some appointments to be made and some research of a trial nature to be started. A number of other countries have acted somewhat similarly. Czechoslovakia, for example, in addition to financing its own planning phase, has provided from its own funds the office and staff for one of the international conveners, Prof. Malek, who is based in Prague.

In order to plan and carry out national programmes, many countries are setting up their own national committees, often with sub-committees equivalent to the international sectional committees of SCIBP. Thus the United Kingdom, through its committee structure which operates under the patronage of the Royal Society, brings about 150 British scientists into the active planning stage. The United States now has a national committee under Dr. Roger Revelle, Poland one under Prof. Petrusewicz; Japan not only has a national committee but has already submitted a programme. I believe that before long a major problem of SCIBP and of its Central Office will be, not so much to get IBP in motion which has been a preoccupation up until now, but to prevent it running away from itself by becoming too large and unmanageable.

Conclusion

The gestation period of the Programme has been long, but recent progress is at a much greater rate than we had dared to hope a couple of years ago, for the world needs an IBP. What, then, is the philosophy behind it? What is the driving force that causes many busy people about the world to devote their time and energy to helping it on? I suggest it is a spirit of service to our fellow men and to all the other animals and plants that make up the very varied living communities of the Earth. Even in these days of rather selfish approach to living, apparent in the political, commercial and even scientific spheres, I suppose there is some feeling of service in all of us, even if it is only an instinct towards conserving the race, after conserving the individual. Here in IBP is the opportunity for very many biologists, whose efforts may have been not perhaps of very high value to others, to bring their researches together, to focus them and to make them more useful. Here in IBP is the opportunity of showing the world the value of field biology as well as laboratory biology. New resources will undoubtedly be discovered, and so also will be the aesthetic as well as the scientific and economic value of plants and animals in their natural and also in their man-made environments.

An important part of the service IBP hopes to render will be to ensure that at least samples of these wonderful creations of evolution, so different and yet so similar in the several bio-geographical zones, will continue and flourish for subsequent generations of humans to appreciate and use. Nor should we forget the many and diverse races of man himself, with their separate and particular attributes. Unless we understand these matters and use our understanding to plan the future, they will go down before the axe and fire, the bulldozer or the hypodermic needle. This marvellous biological differentiation on the Earth would then tend towards dull uniformity and even obliteration.

OBITUARIES

Dr. L. H. Gray, F.R.S.

Dr. Louis Harold Gray, first director of the British Empire Cancer Campaign Research Unit in Radiobiology, died suddenly on July 9, 1965. He was fifty-nine.

Hal Gray, as he was known to his friends the world over, enjoyed a unique position in his chosen subject. Radiobiology calls for a wide-ranging mind, able to grasp the fundamental concepts of physics, chemistry, biology and medicine. To the end of his life, Gray retained an intellectual vigour which enabled him to seize the essentials of a new scientific discipline, and relate them to the existing body of knowledge.

Educated at Christ's Hospital, Horsham, to which he won a scholarship, he soon displayed an outstanding aptitude for science and mathematics. At school he was much influenced by "Chas." Browne, then head of science, who educated his pupils on the principle that it is better to do an experiment than to look up the answer in a

book. This 'do it yourself' approach was to remain a characteristic feature of Gray's work throughout his life.

From school, he won an Exhibition at Trinity College, Cambridge, and two years later took Part I of the Tripos in physics, chemistry, mathematics and mineralogy, and was awarded first-class honours. Proceeding to Part II in physics, he again achieved a first and was able to commence research at the Cavendish Laboratory during the exciting period of Rutherford's professorship. In 1930 he took his Ph.D. and received a Fellowship at Trinity.

His work at this period was concerned with the scattering and absorption of γ-rays, and in his first publication (1929) he enunciated the cavity ionization principle, which had previously been described by Bragg in 1912, although Bragg's work was unknown to Gray at the time. This theory, which is fundamental to γ-ray dosimetry, became known as the Bragg–Gray principle.

Despite his success in pure physics, he began to find the subject not completely satisfying, and he felt the need to do something of more obvious value for his fellow men. An offer to come to Mount Vernon Hospital as physicist appealed very strongly to him and he took up the position in 1933. Although his work at Mount Vernon was primarily concerned with the accurate measurement of ionizing radiation, he began to take an interest in biological problems under the guidance of that remarkable man, Dr. J. C. Mottram. Before leaving Cambridge, he had begun a fruitful collaboration with F. G. Spear, of the Strangeways Laboratory, studying the effect of neutrons on hen eggs. The neutron source available to them proving to be too weak, Gray, with the collaboration of John Read, built a highly effective neutron generator for an outlay of some £600, with which the first biological results were obtained in 1938. At this time, Read and Gray suggested a unit, the Energy Unit, which was applicable to all forms of ionizing radiation and was almost identical with the rad unit adopted by the International Commission on Radiological Units and Measurements some fifteen years later. During the Second World War, when he was working alone, he began to consider the dynamics of cellular population changes underlying the gross changes in behaviour of a bean root following irradiation, thus anticipating one of the focal points of interest in modern radiobiology.

In 1946 Gray went to the Medical Research Council Radiotherapeutic Research Unit, as senior physicist, and in 1950 was promoted to deputy director. Here, he had overall responsibility for building up several teams to carry out ambitious projects in radiation research, the construction of a cyclotron, the development of radioisotopes in research, and general radiobiology. This heavy administrative load left little time for his personal researches, but his wise choice of staff is reflected in the advances in radiobiology made by his group. The classic investigations of Howard and Pelc on the relation of DNA synthesis to the mitotic cycle, and of Miss Alper on bacteriophage, date from this period.

In 1952 Dr. Gray initiated a programme of research on the oxygen effect in radiobiology, and its relation to radiotherapy. Those who had the good fortune to work under Hal Gray at that time will always remember his boyish and generous enthusiasm, which infected all those near to him. Within six months the main outlines of the picture had been established—a picture which still inspires much of present research in radiobiology. It was characteristic of Hal's disarming modesty that his collaborators had the greatest difficulty in persuading him to be the first author of the paper which recorded this work.

In 1953 Gray resigned his position at Hammersmith, following disagreements on scientific policy. Thanks very largely to the good offices of the late Lord Horder, who placed great faith in Gray, the British Empire Cancer Campaign undertook to finance a new Unit at Mount Vernon, where he would be director with freedom to pursue his own lines of research.

With considerable foresight, Gray planned the use of a linear accelerator for the development of pulse radiolysis methods, in collaboration with J. W. Boag, a technique which has already revolutionized radiation chemistry. At the same time, he gathered around him a small group whose basic training lay in physics, chemistry, biochemistry, biology and medicine, since he believed strongly that collaborative studies by such a group offered the best hope for advance in an inter-disciplinary field. It was also a great delight to him to return to experimental work, which he did with undiminished energy, planning experiments which made few concessions to human requirements for sleep and food.

He had no love of committees, but he bravely accepted the administrative burdens which were placed on him. His work for the International Commission on Radiological Units will be particularly remembered, and the personal effort which he put in as president of the second International Congress of Radiation Research had a lot to do with the acknowledged success of that meeting.

Gray received many honours in recognition of his work, but two he prized highly were American awards, the Judd Award in 1954 and the Bertner Foundation Award in 1964. He was elected Fellow of the Royal Society in 1961 and received an Honorary D.Sc. at Leeds in 1962.

No catalogue of publications or prizes gives a true picture of Hal Gray's contribution to radiobiology. He delighted in helping other workers, particularly the younger ones, and he never grudged the time devoted to giving help and advice. Even an anonymous referee's comment would engage the whole of his great powers of critical appraisal and constructive comment. Varied and remarkable as his contributions were, it is certain that he will be remembered by those who knew him, not only as a great scientist, but also as a great man.

O. C. A. Scott

Dr. O. S. Sinnatt

Dr. Oliver Sturdy Sinnatt, professor of aeronautical science in the Royal Air Force College, Cranwell, from 1920 until 1940, died on May 28, 1965, at the age of eighty-two. Dr. Sinnatt was born in Liverpool on September 6, 1882, and educated at the College of Technology, Manchester, and the Owens College (later University of Manchester). He graduated B.Sc. in 1904, M.Sc. in 1907, D.Sc. (London) in 1919, and was elected Associate Fellow of the Royal Aeronautical Society in October 1924. After a year as master of engineering at the Carpenters' Technical Institute, Stratford, he joined the staff of King's College, London, in 1905 as a demonstrator, becoming a senior demonstrator and then a lecturer in the Department of Mechanical Engineering.

During the early part of the First World War he held various posts with the University of London Officers' Training Corps; for a period he was in charge of the Training Camp, Perivale. He was afterwards transferred to the London Regiment and posted to France early in 1917, rising to be second in command of the 2/2 Battalion of that Regiment.

He was severely wounded at Poelcappelle in 1917 and awarded the Military Cross in 1918. He joined the Air Ministry as a technical officer (Instrument Section) in 1918. He was appointed professor of aeronautical science at the Cadet College, Cranwell, in 1920.

In 1940, Dr. Sinnatt was appointed to the School of Aeronautical Engineering, Henlow, as senior education officer, where he remained until his retirement in 1942.

In 1920 he married Marjorie Helen, the only daughter of the late W. R. Randall, by whom he had two sons, the elder of whom was killed in action in 1944.

C. E. P. Suttle

NEWS and VIEWS

Director of Building Research, Ministry of Technology : Dr. Frederick Measham Lea, C.B., C.B.E.

Dr. F. M. Lea relinquishes his duties as director of building research in the Ministry of Technology on December 31, having reached retirement age. During his twenty-year tenure, the work of the Building Research Station has been greatly extended in scope, now ranging from research on the use of materials to structures, soil mechanics, heating, ventilating, lighting and acoustics, mechanical plant for building, operational and economics research and studies of user needs. Although often accused of technological backwardness, the industry has, in fact, made very rapid application of much of the work of the Station, particularly in the newer fields of research on mechanical plant and on costs and economics. In addition to this national activity, Dr. Lea has taken an active part in international collaboration in building research and he has served as president or as a member of council of most of the organizations working in this field. His early work at the Station, before appointment as director, was on the chemistry of cement and concrete and he has throughout retained a special interest in this field. It has attracted to him a number of awards, culminating in the Walter C. Voss Award of the American Society for Testing Materials, in 1963. His text-book *Chemistry of Cement and Concrete* is an established source of reference. He became a Fellow of the Royal Institute of Chemistry in 1936. He was appointed O.B.E. in 1944, C.B.E. in 1952 and C.B. in 1960, elected an Honorary Associate of the Royal Institute of British Architects in 1947 and an Honorary Fellow of the Institute of Builders in 1965.

Dr. J. C. Weston

Dr. J. C. Weston has been appointed director of the Ministry of Technology's Building Research Station at Garston, Watford, Hertfordshire, in succession to Dr. Lea. The appointment takes effect from January 1, 1966. Dr. Weston joined the Building Research Station in 1947 as a principal scientific officer, and was promoted to senior principal scientific officer in 1953. His concern was with building production methods and costs. Since 1959 Dr. Weston has been, as a deputy chief scientific officer, group head of the Station's Building Operations and Economics, User Requirements and Mathematical Services Divisions. He has been on secondment to the National Building Agency as chief executive of the Operational Division since June 1964. Dr. Weston was educated at Nottingham and obtained his degree at London and his Ph.D. for work on temperature changes accompanying magnetization processes in ferromagnetic substances. During and immediately after the Second World War, Dr. Weston was with the Admiralty Scientific Service. He was engaged in work on torpedoes and mine detection. This was followed by a year at The Plessey Company, Ltd., where he was responsible for the Physics Research Laboratory.

Director of the Scottish Plant Breeding Station : Dr. J. W. Gregor, C.B.E.

Dr. J. W. Gregor retired on September 30 from the post of director of the Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian. After taking his Ph.D. at the University of Edinburgh in 1925 he joined the staff of the Station as chief assistant. In 1939 he was awarded a D.Sc. and was appointed director in 1950. He was made a Fellow of the Royal Society of Edinburgh in 1957 and was awarded a C.B.E. in 1961. Internationally known for his experiments on the genetics of wild populations of *Plantago maritima*, he endeavoured to show the agricultural significance of his genecological studies by relating them to pasture improvement in the Scottish hills. Always interested in the nomenclature of plants, he has made a number of contributions to the literature on ecotypic differentiation and the recognition of intra-specific variation in plants. Among many consultative groups on which he served was the International Commission for the Nomenclature of Cultivated Plants which produced the *International Code of Nomenclature*. Dr. Gregor has been succeeded by Mr. N. W. Simmonds.

Mr. N. W. Simmonds

Mr. N. W. Simmonds, head of the Department of Potato Genetics at the John Innes Institute, Hertford, has been appointed to the post of director of the Scottish Plant Breeding Station, in succession to Dr. J. W. Gregor. Mr. Simmonds was educated at Whitgift and Downing College, Cambridge, and then went to the Imperial College of Tropical Agriculture, Trinidad, in 1944 under a Colonial scholarship. After holding a lectureship in botany at the Imperial College of Tropical Agriculture, Trinidad, he became senior cytogeneticist in the Banana Research Scheme and Regional Research Centre; a new tetraploid variety of banana was produced by the Scheme during his tenure of the post. In more recent years he has been consultant in sugar-cane breeding in the West Indies. He returned to Britain in 1959 to become head of the Department of Potato Genetics at the John Innes Institute. Mr. Simmonds's botanical and plant-breeding investigations have taken him on extensive travels in Europe, Asia, Africa and America, and, while his main interests lie in the cytogenetics and evolution of crop plants (especially of bananas and potatoes), he has also published widely in the fields of ethnobotany, systematics and phenol biochemistry.

Psychology in the University of Reading : Prof. Magdalen D. Vernon

The retirement of Prof. M. D. Vernon from the chair of psychology in the University of Reading constitutes a landmark not only in her own working life, but in the development of the subject in Britain. She started work in Sir Frederic Bartlett's laboratory at Cambridge in 1921 as an Industrial Health Research Board investigator at the beginning of the remarkable period of progress there between the two Wars. She remained—later as a Medical Research Council staff member—until 1946, when the late Prof. A. W. P. Wolters secured her services as a lecturer at Reading. Appointed senior lecturer in 1955 and reader in 1956, she succeeded Prof. R. C. Oldfield in the chair in that year. Her massive contributions to the study of visual perception and especially of the processes involved in reading, both in experimental papers and in books such as *Visual Perception*, had already earned her an international reputation before she went to Reading. During her twenty years there that reputation has much increased, while her work in other fields, such as the motivation of children, has widened its basis. Her unfailingly high standards in research and in teaching have been a spur to her colleagues and students alike, and if many perhaps have found them difficult to emulate, much excellent work and an acknowledged distinction have been their fruit. Prof. Vernon's astringent good sense on academic and other committees has always been effective and deeply appreciated by her colleagues. Her period at Reading has spanned a time of very considerable expansion of the subject, with a large increase

in student numbers and in research activities. As elsewhere, this has entailed a long struggle with the difficulties of inadequate accommodation and staff recruitment. But her admirable management and persistent advocacy leave the Department with a greatly enhanced reputation, and well poised for the further developments which may confidently be expected under her successor, Dr. M. Treisman (see *Nature*, 207, 689; 1965).

Discoverer of Nickel: Axel Fredrick Cronstedt

Axel Fredrick Cronstedt, the Swedish chemist and mineralogist, who died on August 17, 1765, is remembered not only for his discovery of nickel but also for his researches and writings. Though a member of the Stockholm Academy of Sciences, he had no university training; for, as a student at Uppsala, lack of finances meant abandoning his academic career to take a post as metallurgist in the Swedish Department of Mines. Yet this proved beneficial both to him and to his country. Georg Brandt coached Cronstedt in the techniques of assaying. In 1751 Cronstedt came across a new mineral from a cobalt mine, which at first was thought to contain copper, yet which yielded none by displacement from its solutions. The mineral, now known as niccolite, or nickel arsenide, formed green crystals on weathering; it was from these that Cronstedt prepared an oxide which on reduction with charcoal gave a new metal. Only in 1754 did Cronstedt christen it nickel, and describe the silver-white and feebly magnetic regulus to the Stockholm Academy. Cronstedt noted the brown borax bead, the blue solution formed with ammonia, and the close resemblance to cobalt. Chemists in France in particular refused to accept the new metal until Torbern Bergman, in 1774, added further evidence by preparing a specimen of it free from the cobalt, arsenic and iron which had contaminated Cronstedt's first specimen. In the history of chemistry and metallurgy Cronstedt's name appears frequently in connexions other than nickel, being listed with platinum, iron ores and zinc extraction—a field in which Cronstedt collaborated with Rinman, whose name remains in 'Rinman's Green'. Cronstedt won a niche even if only for his *System of Mineralogy* translated into several languages.

Political and Economic Planning

The annual report of Political and Economic Planning (P.E.P.) outlines the current research programme which, during 1964–65, focused on problems of planning and policy-making in economic policy, Government, management, social issues and international affairs (Pp. 12. London: Political and Economic Planning, 1965). The Social and Economic Archives Committee has entrusted to P.E.P. the establishment of a Survey Archives Centre in Britain; for an initial period of two years P.E.P. will collect and disseminate information about survey data held, and surveys planned by university departments, research institutes, market research and commercial organizations and other relevant problems. During the year, a study of the effects of imprisonment of a man on his wife and dependants was published under the title *Prisoners and their Families* (see p. 216 of this issue). A survey of East African students in Britain was also completed during the year and published under the title *New Commonwealth Students in Britain—with Special Reference to Students from East Africa*. Since 1931, P.E.P. has organized 500 broadsheets and 50 books; a list of publications issued during 1963–65 is appended to the annual report.

Energy Forecasts for Britain

Mr. D. G. Tipping in the *Westminster Bank Review* for August 1965 questions the forecast of energy demand for 1980 of 450 million coal equivalent tons as too high, since it is based on the assumption of a 4 per cent growth in the economy. He suggests that a more realistic range of expectation for 1980 would be 400–420 million tons, but he does not think that Britain is likely to be faced with a sudden critical shortage of energy brought about by unexpected changes in demand. He considers that the output of 200 million tons of coal per annum advocated by the coal industry is unrealistic, and points out that the demand for coal from the gas industry is likely eventually to fall to zero. The one market for coal which is likely to grow considerably is for the generation of electricity, and the main uncertainties here are the proportion of future demand to be met by nuclear power and the balance to be struck between the conventional fuels. There seem to be three major ways in which the coal industry could be helped to play its part in a cheap energy policy: through capital reorganization, apportionment of social costs (which lie at the core of the problem), and through the pricing system. Mr. Tipping insists that the State and not the consumers should bear the burden of interest on over-capitalization of some parts of the industry. Social costs should be borne by society and also a more rational pricing policy should be introduced. Any rational pricing system is logically prior to a rational target. He points out that we must plan not only to minimize any error but also to introduce sufficient flexibility to overcome the effects of error. There is already considerable spare primary capacity because the coal is accessible and merely needs the men and machines to get it. We are unaware, however, of the physical and economic possibilities of mothballing pits, or of the extent to which the present surplus capacity of 40 million tons would match the capacity of loss-making pits which one would like to close.

Utopia Reviewed

The Spring, 1965, issue of *Daedalus*, the journal of the American Academy of Arts and Sciences, consists of a series of essays on various aspects of Utopia by writers who if not American born are, with two exceptions, working in the United States. Some, like Lewis Mumford's "Utopia, the City and the Machine", Prof. F. E. Manuel's "Toward a Psychological History of Utopias", Prof. Northrop Frye's "Varieties of Literary Utopias" and Judith Shklar's "The Political Theory of Utopia", have a certain historical bias. Others, including two at least of those already mentioned, are more concerned with the social implications, for example, Prof. C. Brinton's "Utopia and Democracy", Prof. A. B. Ulam's "Socialism and Utopia", F. Bloch-Lainé's "The Utility of Utopias for Reformers", Bertrand de Jouvenal's "Utopia for Practical Purposes" and J. R. Pierce's "Communications Technology and the Future". These, and also Prof. P. B. Sears's "Utopia and the Living Landscape" and Prof. J. M. Smith's "Eugenics and Utopia", may appropriately be read in conjunction with Jacques Ellul's recent book *The Technological Society*, to the fatalism of which some of the essays apply a slight corrective. Not even the more historical essays, however, refer to Marie Louise Berneri's scholarly work *Journey Through Utopia*, published posthumously in 1950, to which Mumford himself paid a generous tribute at the time. Prof. Frye, in fact, provides no references at all and generally, in contrast to Marci Berneri's book, the bibliographical side of the essays is weak. The titles of such essays as those of de Jouvenal, Sears, Smith and Pierce indicate their topical character, and perhaps the most optimistic are those of Prof. G. Kater on "Utopia and the Good Life" and Prof. Maren Lockwood on "The Experimental Utopia in America": both of these essays, incidentally, are well provided with references. While Mumford himself has not lost the optimism that marks his well-known trilogy, in the essay in *Daedalus* he accepts much of Ellul's position as to the dominance of the machine and points out that we can no longer think of the components of technology as additive.

We must face the implications of an articulated totalitarian system of which the machine is god and must recognize that our present scientific methodology is inadequate to deal with every aspect of human experience.

Social Implications of Automation

Dr. A. Hovne, in a recent issue of *Impact of Science on Society* (15, No. 1; 1965), discusses some social implications of automation, including the reasons which are likely to be decisive for the introduction of automation and why a new technique may not be adopted. After considering the unemployment aspects, he deals with the implications for four selected aspects of social life; for work in automated enterprises; for their organization; for the organization of Government. There are also certain broad choices to be made, particularly those relating to the place of work in the life of men, mobility in employment, the content of education, and to study as work. The two Cantor Lectures given to the Royal Society of Arts last March by T. R. Thompson and now published in the *Journal of the Royal Society of Arts* for August 1965 also deal with automation. The first of these lectures considers the economic problems of introducing automation into any process and the problem of finding the people to plan automation systems. The second lecture, dealing with the social consequences of automation, considers the need for anticipating its effects and the character of the new society in which automation is widely used. Besides the planning for this future, there is the problem of ensuring that the plan is feasible and also the further problem of providing the type of labour required. Finally, Mr. Thompson stresses the need to educate people to fit them for such a future and the importance of minimizing the effects of change. He suggests that a new emphasis should be placed on the real purpose of economic change; we must ensure that automation is made to serve our deepest needs, to give more leisure and to make work more interesting, so that we find greater contentment and happiness.

Research in Human Genetics and Studies of Twins

The World Health Organization, in recognition of the increasingly important part that genetic studies are playing in the medical sciences, has established at its headquarters in Geneva a Human Genetics Unit. The purpose of the Unit is to co-ordinate and stimulate research and training in human genetics. Dr. R. L. Kirk, who is in charge of the new Unit, was formerly reader in human genetics in the University of Western Australia, where his research was concerned mainly with population genetic studies in isolated groups in south and south-east Asia, Australia and the Western Pacific. Current activities of the new Unit in Geneva include investigations of populations of unusual genetic interest, study of various hereditary conditions such as haemoglobinopathies, the setting up of facilities to service research projects, such as international reference centres for genetic markers, and improvement and standardization of methodology in genetic studies. The Unit hopes to give increasing attention to research on the distribution and public health significance of the haemoglobinopathies, including the thalassaemias and glucose-6-phosphate dehydrogenase deficiency, as well as to stimulate interest in new methods of treatment for cases of homozygous sickle-cell disease and thalassaemia.

The use of twins in epidemiological and genetic research is being evaluated at the present time, and the World Health Organization hopes to act in future as a clearing-house for information on twin studies. It also hopes to play an important part in co-ordinating population genetic studies, particularly those concerned with populations still having a food-gathering or simple agricultural economy, or living as isolates in more highly developed communities. Future activities will be directed toward the role of genetic factors in mental disorder, the standardization of techniques in studying chromosome aberrations associated with spontaneous abortion, the study of somatic cell genetics and the genetic consequences of exposure to novel chemicals in the human environment.

Portraits of Tasmanian Aborigines

Although the aborigines of Tasmania have been extinct for some decades, a number of portraits of them are in existence which are of considerable anthropological interest. An account of them, the circumstances in which they were produced and passed into the hands of various collectors, together with their present whereabouts, has recently been published (*Records of the Queen Victoria Museum, Launceston.* New Series, No. 18: *Thomas Bock's Portraits of the Tasmanian Aborigines.* By N. J. B. Plomley. Pp. 24 (10 plates). Launceston: The Queen Victoria Museum, 1965.) The portraits are in water-colour. The originals are almost certainly the work of Thomas Bock, an engraver who was transported to Tasmania in 1824, and they were made at the request of G. A. Robinson, who was engaged on expeditions of conciliation with the aborigines at that time. Duplicates were also produced by Bock as well as by his son, Alfred Bock, along with other artists, mainly for Lady Franklin, wife of the Governor General. Later, many of the pictures found their way into the hands of collectors, like Dr. Joseph Barnard Davis, a surgeon living at Shelton, Staffordshire, and Richard Cull, who was secretary of the then Ethnological Society. Now, most of the portraits form part of permanent collections at the Pitt-Rivers Museum, Oxford, the British Museum, the Royal Anthropological Institute, the Fuller Collection (London), the Tasmanian Museum, and the Queen Victoria Museum, Launceston, Tasmania. Some of them were housed in the Crystal Palace, and were destroyed by the fire in 1936. The paper contains twenty reproductions of the portraits in black-and-white. On aesthetic grounds alone they are of outstanding merit; and while it is now impossible to judge of their quality as likenesses, they are impressively life-like, constituting a record of the characteristics and facial expressions of a group of people which is compelling and unique.

The Great Barrier Reef, Australia

The Editor has received the following communication from Dr. O. A. Jones, chairman of the Great Barrier Reef Committee, Australia: "I would like to draw the attention of your readers to the greatly expanded facilities for research on the Great Barrier Reef, Australia. The Great Barrier Reef Committee has recently spent nearly £30,000 on providing living accommodation and additional laboratory space and equipment. The facilities might be described as rather more than basic, in that they include, in addition to collecting gear and preserving materials, such items as a fume cupboard, microscopes, centrifuge, mettler balances and an incubator. There is fresh and salt water on tap, gas for laboratory heating and cooking, refrigeration (both laboratory and domestic), electric light and power, aquaria and two small boats. Scientists from anywhere in the world may live in reasonable comfort at the Station and carry out research programmes, short or lengthy, of their own choice in any of the scientific disciplines applicable to barrier reefs. The Station is admirably situated for carrying out sedimentation and beach rock studies as well as marine biology. Heron Island Reef is about twelve square miles in area; neighbouring Wistari Reef is much larger, and there are nineteen other reefs within an area of forty square miles. The fauna of Heron and Wistari Reefs is protected by law, but scientists may collect specimens for their research under Government permits. Additional information is obtainable from the honorary secretary, Dr. R. Endean, Department of Zoology, University of Queensland, Brisbane, Australia."

International Conference on Mechanisms and Machines

During September 27–October 1 the first international conference to be held in Bulgaria took place at Drouzhba resort, near to Varna. Delegates from Canada, the United Kingdom, Australia, Czechoslovakia, both German Republics, Hungary, Italy, Poland and the U.S.S.R. were present, and the topics under discussion ranged from the determination of the instantaneous screw axes in spatial mechanisms, and from non-linear problems of centrifugal pendulum vibration absorbers to the design of electronic computers and of high-speed input–output devices. The levels of presentation and of technical content of all the papers were high; this, together with the excellent organization of the conference, and the really beautiful situation of the hall—not 20 yards from a sandy beach and the Black Sea—made the event one which will be long remembered with pleasure by those who were present. An important outcome of the meeting has been the setting up of an informal communications system for the interchange of information in a field which is of interest and importance—but as yet not provided with a recognized journal.

'Clean Room' Technology

A conference was held at the Technische Hochschule, Stuttgart, during September 6–7, under the chairmanship of Dr. R. Kratel. About a hundred scientists and engineers attended, most of the main German firms engaged in ventilation and air filtration being represented. A message was received from the American Association for Contamination Control. The first day was devoted to the physics of aerosols. Dr. C. N. Davies gave a general account of the deposition of airborne contamination by diffusion processes and by mechanical effects from air at rest and in laminar and turbulent flow. Natural aerosols in the atmosphere were described by Dr. K. Bullrich of Mainz University. Two papers followed on the analysis of aerosols, Dr. Gahm, of Zeiss, giving a complete analysis of limiting factors in visual microscopy, and Prof. R. Hodkinson, of Virginia State College, discussing the performance of automatic optical dust counting instruments in the light of the Mie theory of light-scattering and recent research on the effect of varying design factors, such as angle of acceptance. During the second day, problems of immediate practical application were dealt with. Dr. D. Hasenclever, of Staubforschungsinstitut, Bonn, gave an analysis of filter performance, and J. R. Weersing, of Royco Instruments, dealt with recent findings on the performance of his firm's particle counter which was developed particularly for use in factories making components for missiles in the United States. The need for dust-free work-places is strongly felt in the electronics industry, and some typical problems in International Business Machines Corp. were explained by Dr. Lechler. The conference concluded with an address by Dr. Kratel, who considered the correct lines for industrial development and the question of standards of aerosol concentration and particle size.

American Society of Plant Physiologists : Awards

Prof. Frits W. Went, of the University of Nevada, has been awarded the Charles Reid Barnes Life Membership Award of the American Society of Plant Physiologists. Each year the Society confers this distinction on a senior member of the profession in recognition of his character and contributions to scientific research. Prof. Went is recognized for his broad contributions to the field of botany and for his pioneering research on the bioassay of plant hormones (auxins). His work laid the foundation for the agricultural success of synthetic weed killers, fruit drop inhibitors, and the many other practical applications of these substances. Prof. Went was also concerned with pioneering research in the quantitative study of the effect of weather on plants. This culminated in his development of the air-conditioned greenhouse (phytotron) which has led to the plant growth chambers that are now part of the equipment of every major botanical laboratory. His manifold other interests include investigations of the formation of atmospheric smog from plant volatile exudates, the interrelationships of various plant species with each other, the discovery of many of the properties of plant hormones, and the physiology of desert plants. In addition to his scientific research, Prof. Went has achieved recognition through his effective lecturing, inspiring teaching and competent administration, which have been recognized through professorships at the California Institute of Technology, and the Washington University, St. Louis, and the directorship of the Missouri Botanical Garden.

Officers

The following have been elected officers of the American Society of Plant Physiologists: *President*, Prof. A. Carl Leopold (Purdue University); *Vice-President*, Prof. Robert S. Bandurski (Michigan State University); *Secretary*, Prof. Robert S. Loomis (University of California at Davis). Prof. Kenneth V. Thimann, of the University of California at Santa Cruz, has been elected to the Executive Committee.

University News: Birmingham

The following appointments have been made: *Lecturers*, Dr. A. J. Biddlestone (chemical engineering); Dr. I. L. Dillamore (physical metallurgy); D. E. Evans (physics); Dr. A. W. Nicol (mining and minerals engineering); Dr. N. M. Queen (mathematical physics); Dr. K. H. Star (psychology); *Senior Research Fellow*, Dr. J. Stephen (microbiology); *Research Fellows*, B. E. P. Beeston, M. D. Hunt, M. Norman and R. J. M. Willcox (physical metallurgy); Dr. M. Cordey-Hayes (physics); Dr. R. Haque (mining and minerals engineering); M. A. S. Sweet (electronic and electrical engineering); D. R. Whitehouse (chemistry); R. C. Woodman (engineering production); Dr. A. J. Collings (medical biochemistry and pharmacology).

Announcements

Dr. Sigvard Eklund (Sweden), director general of the International Atomic Energy Agency since December 1, 1961, was reappointed for a second four-year term of office at the Agency's General Conference in Tokyo on September 27.

A symposium on "Subcellular Movement", arranged by the American Society for Cell Biology, will be held in Philadelphia during November 10–12. Further information can be obtained from Professional Associates, 6520 Clayton Road, St. Louis, Missouri.

An international symposium on "Volcanology", sponsored by the International Association of Volcanology of the International Union of Geodesy and Geophysics and organized by the New Zealand Geological Survey, will be held in Rotorua and Wellington, Auckland, during Novemaber 22–December 3. The two particular subjects proposed for the symposium are: rhyolites, ignimbrites and pumice, and associated volcanism; geothermal resources with particular reference to the origin, geology, geophysics and geochemistry of hydrothermal systems of possible economic importance. Further information can be obtained from the Secretary, International Symposium on Volcanology, P.O. Box 499, Rotorua.

Erratum. In the article entitled "Light-induced Stomatal Opening and the Postulated Role of Glycollic Acid", by Prof. O. V. S. Heath, Dr. T. A. Mansfield and Dr. Hans Meidner, which appeared on p. 960 of the August 28 issue of *Nature*, in column 1 line 13 from bottom on p. 961 '1·34' should read '$\bar{1}$·34' (that is, a lower log stomatal resistance in blue than in red light).

THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY

THE fifth annual report of the Council of the Institute of Physics and the Physical Society * covers the period ending on December 31, 1964, and incorporates details of the activities of the various committees, branches and groups of the Institute and Society, together with the financial statement and account for the year. The report was presented and adopted at the annual general meeting held on July 6, 1965, at the headquarters of the Institute and Society, 47 Belgrave Square, London.

During the year under review, 970 applications for election or transfer to the various grades of membership were received, compared with 1,165 in 1963. The total membership of 9,750 on December 31, 1964, consisted of 1,416 Fellows, 3,558 associates and 2,444 graduates of the Institute and 1,256 Fellows of the Physical Society, in addition to students and subscribers. Although this represents a net increase of 280, the rate of growth has declined during the past two years, and the Council in its report expresses considerable concern. It is known that many physicists qualified to become members attend the scientific meetings and conferences organized by the Institute and Society, but nevertheless do not apply for membership. Various methods to improve recruitment are under active consideration by the Membership and Education Committee. A new grade of membership, Licentiate, intended for assistants in laboratories possessing a knowledge of physics not quite so extensive as that required for graduateship, and for certain teachers in schools and technical colleges, became operative at the beginning of 1965.

Representatives of the Institute and Society visited five technical colleges, and the applications of three of these colleges for recognition or extension of recognition for the purpose of the membership regulations were approved. Sixty-two technical colleges presented 892 candidates for the Ordinary National and forty-one colleges 614 candidates for the Higher National Certificate in applied physics; 589 and 418 respectively were successful. Examinations for the Higher National Diploma in applied physics were held for the first time during 1964. Twelve of the thirteen candidates entered by two colleges were successful. The seven question papers set in Part 2, and the four compulsory papers and the general paper in Part 1 of the graduateship examination were published in the October issue of the *Bulletin* of the Institute and Society, and also as a separate booklet. Of the 97 candidates for Part 2, 27 passed (two with honours), and of the 79 for Part 1, only 12 were successful.

An enquiry conducted by the Membership and Education Committee revealed that 1,597 students were admitted at the beginning of the session 1964–65 to the 1,907 available places in the physics departments of the universities (excluding the University of Cambridge) in England and Wales. The corresponding figures for 1963 were 1,524 and 1,708 respectively. A report on the survey of salaries and emoluments received by the members of the Institute as at August 1, 1964, was published in the January 1965 issue of the *Bulletin*. This was the sixth survey carried out by the Institute and the first since the amalgamation of the Institute and the Society. A subcommittee of the Membership and Education Committee is now completing a revision of the first draft of a statement on professional conduct which deals mainly with terms and conditions of employment and consulting work. The revised draft is to be printed and submitted to members for comment and revision before adoption.

The Institute and Society were responsible for the organization of the international conference on magnetism held during September 7–11, 1964, at the University of Nottingham. More than 500 attended the conference and some 230 papers were presented. The text of the 1963 Guthrie Lecture, on the subject of magnetic processes in weak and moderate fields, which was delivered by Prof. L. F. Bates on September 9 during the conference, has now been published (*Proc. Phys. Soc.*, 84, 625; Nov., 1964). The lecture had been specially postponed so that Prof. Bates might deliver it in his own Department at Nottingham. Many other conferences and symposia were organized and held during 1964, both by the Meetings Committee and the Nuclear Physics and Solid-state Physics Sub-committees of the Institute and Society and by the twelve branches and eleven specialist subject groups. Details of these are given in the annual report.

The 1964 Guthrie Lecture, which was delivered by Prof. M. Ryle on October 1 during the autumn conference of the Electronics Group at the University of Keele, was entitled "Radio Telescopes" and the text appears in the February 1965 issue of the *Proceedings of the Physical Society* (85, 201; 1965). Dr. P. H. Fowler, who gave the 1964 Rutherford Memorial Lecture on September 9 during the conference on "Low- and Medium-energy Nuclear Physics" at the University of Sussex, spoke on the subject of "Pi-mesons versus Cancer" (*Proc. Phys. Soc.*, 85, 1051; June 1965). Dr. A. R. Lang received the Charles Vernon Boys Prize for his development of the technique of X-ray topography, and Dr. W. Marshall the Maxwell Medal and Prize for his contributions to the theory of magnetism. The presentations were made at the annual dinner of the Institute and Society in London on May 5, 1964. Prof. J. Friedel was awarded the 1964 Holweck Medal and Prize for his work on the electronic structure of metals. The Council has decided that after 1965 the Guthrie and Rutherford Lectures will be replaced by the awards of medals and prizes.

The forty-eighth annual exhibition of scientific instruments and apparatus was held during January 6–9, 1964, in the two halls of the Royal Horticultural Society, London. The *Journal of Scientific Instruments*, in its May 1964 issue, contained several articles and a special survey devoted to equipment displayed at the exhibition. The careful selection of exhibits and the section devoted to educational instruments and experiments were commendable features. The *British Journal of Applied Physics* revised its format to that of the *Proceedings*, but retained the original colour of its cover. A reorganization of subject-matter between the two journals was made. The volume of material submitted to the Institute and Society's three journals continues to increase, but the report directs attention to the growing number of papers which in the form received are unacceptable for publication. The Council had decided to launch a new monthly journal, the *Journal of Physics Education*, principally for sixth-form teachers of physics. In co-operation with other scientific bodies the Institute and Society have urged that closer collaboration be established between editors of physics journals so that by better choice and improvement of articles and by uniformity of presentation the standing and sales of British journals may be enhanced. The *Student Monograph Series* of books, commenced in 1953 and intended for students of the Higher National Certificate in applied physics, has since been widened in scope for undergraduate and graduate students, but since there are now adequate publications of this kind it has been decided to complete the series with the publication of "Subjective Limitations on Physical Measurements" by C. A. Padgham. The volume entitled "Dimensional Analysis and Scale Factors", by R. C. Pankhurst, was published during 1964.

The financial statement refers to the gratifying increase of £13,964 in members' fees and to the increases in the exhibition receipts and in non-members' conference fees. The increase in activities and the general rise in costs

* Report of the Council of the Institute of Physics and the Physical Society for the year 1964. Pp. 25. (London: The Institute of Physics and the Physical Society, 1965.)

combined to raise the total expenditure, and the total reserves at the end of the year of £158,673 still fell short of the aim to obtain a sum equal to the amount required for one year's working.

The report gives prominence to the proposal by the Council in July 1964, and the agreement in December 1964, to purchase the Fulmer Research Institute. The reasons for the bold venture were announced in the February issue of the *Bulletin* when the purchase was made. Not only will the staff at the Research Institute benefit from close association with the Institute and Society, but investment by the Institute and Society in sponsored research, regarded solely as a business venture, will be evidence that the Council is of the opinion that the application of physics to industry is a paying proposition. The purchase was made possible by the generous terms of the owners' offer and by a substantial interest-free loan made to the Institute and Society by Imperial Chemical Industries, Ltd. It is intended that surplus revenue from the Fulmer Institute will be used to help to replace the income at present derived from the seven-year covenants signed by a number of industrial firms in favour of the Physics Trust Fund and which will stop in 1967 and 1968.

S. WEINTROUB

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE National Research Advisory Council, New Zealand, was established by the National Research Advisory Council Act of 1963 to advise the Minister of Science on matters related to scientific research in New Zealand. Its first annual report covers the year ending March 31, 1965*.

At its first meeting in April 1964, the National Research Advisory Council decided to set up 16 working parties to review and report on research and service in various fields. The reports of these working parties cover: (1) *agriculture and forestry*, the dairy industry, field crops, forestry, horticulture, meat and wool; (2) *secondary and tertiary industry*, building and construction, manufacturing and engineering, and transport; (3) *earth and related sciences*, the atmospheric sciences, mineral resources, oceanography, limnology and fisheries and solid earth sciences; (4) *basic sciences*, training and services, atomic energy (scientific services for Government departments and agencies are appended); (5) *manpower, education and training* (this report was deliberately delayed until the working party was able to consider the recommendations of the other working parties and to obtain additional information on manpower).

Meanwhile, the Council is satisfied that the implementation of its present recommendations is feasible and would not be jeopardized by a shortage of trained manpower.

For the year ended March 31, 1965, the estimated expenditure on scientific research and services was about £7·5 million and in addition £827,000 was scheduled for expenditure on new laboratories for Government departments and research associations; the total expenditure represented about 0·48 per cent of the gross national product. Of the £7·5 millions, £6·1 millions was provided on the votes of eleven Government departments and, of this, £661,000 went in grants to other research organizations. These figures probably underestimate the total expenditure on science since no allowance is made for expansion by the Post Office, railways, the Broadcasting Corporation, or for the salaries of university staff, accommodation or services provided from university funds and used in part for research.

Between 1954–55 and 1964–65 expenditure on science in New Zealand increased from £2·73 millions at about 10 per cent annually, and the scientific staff in Government departments increased in the same period from 568 to about 800. The Council suggests that investment in research for an industry should depend on such criteria as the annual value of production; the national importance of the industry; the significance and number of problems affecting the industry; the chances of a solution being found to selected problems, taking into account the amount of existing scientific information required; the calibre of the staff available and the availability of suitable facilities; the application of results, bearing in mind the problem of implementation because of difficulties of liaison and information services and the time-lag in the application of research results.

The Council recommends that every effort should be made to increase the research effort directed towards New Zealand's agricultural and pastoral products, particularly those of the meat, wool and dairy industries, and that over the next five years research expenditure in this area should be increased cumulatively by £320,000 a year. Forestry research also requires expansion, and a cumulative increase in expenditure of £40,000 per year is recommended. Very high priority to research into problems of the transport industry is also recommended, including an expenditure starting at £10,000 a year and rising to £50,000 a year in five years, while a Transport Research Advisory Committee should be set up to advise the Commissioner of Transport on the operations of the proposed Research Unit. An additional £26,000 is recommended for the existing building research units, and increased support for operational research in the Department of Scientific and Industrial Research and other organizations is highly desirable. An increase in the next year of about £3,000, rising to £18,000 in about five years, is suggested, and £50,000 should be granted for implementing the second year's recommendations of the report of the Mineral Resources Committee.

* New Zealand. Report of the National Research Advisory Council for the year ended 31 March, 1965. Pp. 51. (*H.* 41.) (Wellington: Government Printer, 1965.)

COMPARATIVE STUDY OF HOUSING IN BRITAIN, FRANCE AND WESTERN GERMANY

BROADSHEET No. 490, issued by Political and Economic Planning and prepared by E. G. Howes, deals with housing in Britain, France and Western Germany*.

In Britain, building research is financed to the extent of about £900,000 per annum at the Building Research Station, and some £100,000 is spent by the Civil Engineering Research Council; the Minister of Works and Public Building has placed research contracts to the amount of £50,000 with universities. Most building research is carried out privately, but whether this is adequate and whether the information gained is accessible is regarded as very doubtful. In Germany the Federal Government and the Regents devote considerable funds to research and the nationalization of techniques and procedure, and also

* *Planning*, **31**, No. 490 (August 1965): *Housing in Britain, France, and Western Germany*. By Eric G. Howes. Pp. 215–270. (London: Political and Economic Planning, 1965.) 7*s*. 6*d*.

support exhibitions, publications, films and other means of disseminating information (no figures for expenditure, however, are given in the Broadsheet).

For the rest, the Broadsheet concludes that British standards in housing are still high in comparison with those of France, but are being overtaken by those of West Germany. In numbers of dwellings built in recent years (until 1964) Britain has been lagging. Her present need is to step up new building schedules, particularly of dwellings to let, and to demolish and replace slums. Housing policy in Britain has been confused by indecision as to whether the provision of houses should be regarded as a social service, or as a commercial operation. The existence of subsidized and also non-profit housing has almost completely stopped the investment of private capital in dwellings for letting.

While it is proper that local authorities should have the major responsibilities for housing families of all income-levels, it may be argued that subsidies should be reserved for those families that, because of age, lack of earning capacity, or other handicaps, are unable to afford economic rents. In both France and Western Germany the policy is to grant housing allowances to such families and the consequences of adopting such a system in Britain should be explored. In Britain the National Assistance Board pays out rent allowances to 1·5 million families that are eligible for assistance, but the policy elsewhere is to make a clear distinction between housing allowances and public assistance. It is desirable also to diversify the means by which houses for letting at economic rents should be provided: the recently created Housing Corporation should be given every support in its encouragement of cost–rent and ownership societies. There seems to be no point in deterring the private sector from competing in this field.

Adoption of long-term planning policies would enable those engaged in producing dwellings to plan ahead and to concentrate on their work without fear of too frequent change. It is impossible to ignore the success of the German housing problem and the extent to which this is attributable to long-term policies. The efforts of Western Germany to influence the supply and price of building land have not always been fully rewarded, but have achieved some part of their object. Ways and means whereby the British Government could exert an adequate influence have received intensive study, and action is now urgently required. If tax concessions are to be given to some form of saving, like those of Trustee Savings Banks, it is arguable that equivalent concessions should be awarded to Building Societies, the efforts of which are directed exclusively to providing finance for housing. The psychological and material effects of such measures have been clearly demonstrated in Western Germany by the successful revival of the saving habit. Encouragement should be given to the house-building part of the construction industry to rationalize its organization, cut out restrictive practices, intensify training and make the best possible use of the skills and knowledge of its management and work people. The possibility of further curtailment of the apprenticeship period with more intensive training and training on actual work is worth considering. Consideration might also be given to whether it is politic to allow public, commercial and industrial building to outstrip the building of dwellings. Experience in Germany and France suggests that self-building societies, which in a small way have done useful work, could be more vigorously encouraged in Britain.

The Broadsheet considers that the trained personnel of the British building industry are probably of as high a calibre as, if not higher than, their counterpart in other countries, and to perform the task that confronts them they need only the leadership, guidance and encouragement that vigorous Government backing can provide.

INSPECTION OF ALKALI, ETC., WORKS

THE one hundred and first annual report on alkali, etc., works in England and Wales, prepared by the Chief Inspector*, covers the year 1964. It stresses the co-operation of industry with the Inspectorate without which, individually by works, and collectively through research associations, and the like, the task of the Inspectorate would be much more onerous. Certainly without this co-operation the Inspectorate would have to be multiplied several times in size and a department would be needed for specialized techniques of waste gas sampling and analysis, as well as for developing control measures for air pollution. The number of works registered under the Act at the end of the year was 1,995, involving the operation of 3,208 processes, and 11,966 visits and inspections were paid during the year, compared with 10,850 in 1963, including 123 special visits by the Chief and Deputy Chief Inspectors. Of this total, 231 were to, or in connexion with, works not registered under the Act, 46 were concerned with control of radioactive emissions and 37 were to, or in connexion with, colliery spoilbanks. During the inspections 4,563 quantitative analyses were made of gases evolved from processes in operation, compared with 3,433 in 1963, while 746 special samples, compared with 615 in 1963, were taken and submitted to the Government Chemist.

The report also emphasizes the effect of the changing face of industry on the task and responsibilities of the inspectorate; a searching and realistic appraisal of the responsibilities and day-to-day activities of the inspectorate has been made. A historical and technical review of the decline and fall of the traditional sulphuric acid lead chamber process and one of the early days of the copper industry are included.

In the belief that most new contact sulphuric acid plants will burn sulphur of high quality as the raw material, tables have been prepared giving the stack heights for final discharge of waste gases from units or clusters of units making daily 200–2,000 tons of monohydrate. If different raw materials are used it will be a simple matter for inspectors and industry to calculate appropriate chimney heights using the information given and to apply it to the particular permitted loss of sulphur burned. Reference is again made to mist emission from certain contact sulphuric acid plants, and while our knowledge of the reasons for this is still obscure the problem receives the close attention of the Inspectorate (some progress appears to have been made).

The Chief Inspector, under "Alkali, etc., Works and Regulations (Scotland) Acts, 1906 and 1951", records that at December 31, 1964, 263 works were registered under the 1906 Act, and 361 processes were inspected within these works. 622 visits were paid by the Inspectorate on work under the Act or in the interest of preventing air pollution, of which 420 were to premises registered under the Act of 1906.

Further experimental testing of emissions of dust from registered premises was carried out on behalf of the Inspectorate by the two-man team provided on contract by the Department of Scientific and Industrial Research. Thirteen works were covered in 41 visits, and 87 tests were made mostly at the electricity power stations. In general, inspections showed that the provisions of the Act were observed in good spirit as well as with attention to the tests.

* Ministry of Housing and Local Government—Scottish Development Department. One Hundred and First Annual Report on Alkali, etc., Works by the Chief Inspectors, 1964. Pp. iv+76. (London: H.M.S.O., 1965.) 5*s*. 6*d*. net.

INTERACTION OF CEPHALORIDINE WITH PENICILLINASE-PRODUCING GRAM-NEGATIVE BACTERIA

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CEPHALORIDINE (7-((2-thienyl) acetamido)-3-(1-pyridylmethyl) cephalosporanic acid, 'Ceporin'[1]) has been shown to be of value in the treatment of infections caused by Gram-negative bacteria[2,3]. Although many cephalosporins are resistant to hydrolysis by β-lactamases from Gram-positive bacteria[1,2,4,5], there have been several reports[2,6–10] that they are readily hydrolysed by certain Gram-negative bacteria. This destruction has been attributed to an enzyme 'cephalosporinase', although it has seldom been shown to be enzymatic and the end-product has not been identified. In view of these reports, it was decided to investigate the action of organisms known to produce penicillinase (*EC* 3.5.2.6.) on cephaloridine, and the action of cephaloridine on these strains of bacteria. In addition, the relationship between 'cephalosporinase' and 'penicillinase' activities was studied.

The following bacterial strains were used in the investigations: *Klebsiella aerogenes* 1, 43, 83, 366, 370, 373, 402, 407, 414, 415 and 418; *K. ozaenae* 61; *Escherichia coli* 214*T*, 419, *K* 12 (F⁻ *met*⁻) and *TEM*; *Aerobacter cloacae* 15 and 53. All these strains produce penicillinase constitutively. *Proteus morgani G* and 185 and *Pr. rettgeri* 410, which produce an inducible penicillinase, were also used. We are very grateful to Dr. Naomi Datta for supplying *E. coli K* 12 and *TEM*. All the other strains were isolated from clinical specimens at Guy's Hospital and are referred to by their laboratory reference numbers.

Organisms were cultured in infusion broth, collected by centrifugation and disrupted by ultrasonic treatment, as previously described[11,12]. The *Proteus* strains, which have little or no basal penicillinase activity[13], were cultured in sub-inhibitory concentrations of benzylpenicillin as inducing agent before gathering. Such cell-free penicillinase preparations hydrolysed the β-lactam bond in certain penicillins, and it was of interest to determine whether the β-lactam bond present in cephaloridine was also susceptible to hydrolysis by these β-lactamases. The rates of destruction of penicillins and of cephaloridine, brought about by the β-lactamase preparations, were measured either acidimetrically (by the spectrophotometric method), or by the hydroxylamine method. This latter method had to be modified slightly from that previously used[12]: it was found that the hydroxamate of cephaloridine was somewhat unstable, in that it started to decompose after about 5 min; however, the hydroxamate was fully formed in 3 min, and addition of the ferric ammonium sulphate reagent at this time gave a chromogen of maximum intensity. With this modification, satisfactory results were obtained by the use of this assay both for cephaloridine and for penicillins.

Rate of hydrolysis of cephaloridine. Cell-free penicillinase preparations from the various strains were incubated in the presence of cephaloridine (initial concentration 5 mM) at 37° in 25 mM sodium phosphate buffer, *p*H 7·4; the rate at which cephaloridine was destroyed by each preparation was compared with the rates of hydrolysis of benzylpenicillin, ampicillin and 6-aminopenicillanic acid. Results are shown in Table 1, taking the rate of benzylpenicillin hydrolysis as 100 in each case. It can be seen that cell-free extracts of the *Klebsiella* and *Aerobacter* strains destroy cephaloridine at approximately the same rate as 6-aminopenicillanic acid, but less rapidly than ampicillin; the *E. coli* and *Proteus* preparations, however, which destroy ampicillin and 6-aminopenicillanic acid only very slowly, hydrolyse cephaloridine more rapidly than benzylpenicillin under these conditions. The enzymes from the *Proteus* strains hydrolyse cephaloridine many times more rapidly than benzylpenicillin.

Identity of 'penicillinase' with 'cephalosporinase'. The distinction between penicillinase and cephalosporinase activity is not at all clear. Some authors[4,10] claim that cephalosporinases and penicillinases are different enzymes whereas Ayliffe[8] leaves the question open. The following three approaches were used in an attempt to answer this question.

(1) Cell-free extracts of *A. cloacae* 53, *E. coli* 214*T* and 419 and *K. aerogenes* 366 were treated with protamine sulphate to remove nucleoprotein, and the supernatant fractions were gel filtered on 'Sephadex *G*50'; each fraction was then assayed against both benzylpenicillin and cephaloridine. In each case, destruction of the two substrates ran in parallel in each fraction.

(2) Penicillinase preparations from *K. aerogenes* 366 and 373 were exposed to 1·31 mM iodine in 2 mM potassium iodide for 1, 2 or 3 min, at 30°, the reaction being stopped when required by the addition of an appropriate volume of 19 mM sodium thiosulphate. Aliquots were then assayed in parallel against benzylpenicillin and against cephaloridine. In each instance, reduction of the 'penicillinase' activity occurred to precisely the same extent as did reduction of the 'cephalosporinase' activity.

(3) The three *Proteus* strains were cultured in the presence of a range of concentrations of benzylpenicillin, varying from one-half to one-twentieth of the minimum inhibitory concentration. Cell-free preparations were then assayed against benzylpenicillin and against cephaloridine. In the absence of inducing agent, there was no activity against either substrate, but in cultures grown in the presence of benzylpenicillin, induction of 'penicillinase' activity ran quantitatively parallel to induction of 'cephalosporinase' activity, for each concentration of inducing agent.

Thus these data suggest that, in the strains investigated so far, penicillinase and cephalosporinase activities are manifestations of one and the same enzyme.

Affinity of substrates for enzymes. The effect on the rate of hydrolysis of varying the concentration of various

Table 1. Rates of Hydrolysis of Cephaloridine, Ampicillin and 6-Aminopenicillanic Acid by Cell-free Preparations of Penicillinase-producing Gram-negative Bacteria in M/40 Sodium Phosphate Buffer *p*H 7·4

Initial substrate concentration was 5 mM. The rate of destruction of benzylpenicillin is expressed in mμmoles/min/10⁹ bacteria/ml. The rates of destruction for the other β-lactam antibiotics are expressed in terms of mμmoles/min, taking the rate of destruction of benzylpenicillin as 100 in each case

Strain of bacteria		Absolute rate of destruction of benzylpenicillin	% Rates of destruction taking the rate of benzylpenicillin destruction as 100%		
			Cephaloridine	Ampicillin	6-Aminopenicillanic acid
A. cloacae	15	5·4	63	123	20
,, ,,	53	430·0	64	105	15
E. coli	*214T*	54·4	172	—	—
,, ,,	419	10·3	230	—	—
,, ,,	*K12*	1·5	180	—	—
,, ,,	*TEM*	1·6	163	—	—
K. aerogenes	1	10·0	59	210	60
,, ,,	43	10·5	55	232	59
,, ,,	83	1·7	29	163	41
,, ,,	366	3,180·0	65	198	73
,, ,,	370	22·0	45	187	60
,, ,,	373	1,360·0	69	195	77
,, ,,	402	7·6	43	227	68
,, ,,	407	5·4	73	185	55
,, ,,	414	3·8	54	151	48
,, ,,	415	10·3	42	260	77
,, ,,	418	20·6	9	175	55
K. ozaenae	61	22·3	51	200	68
Pr. morgani	G	60·0	306	—	—
,, ,,	185	57·9	1,012	—	—
Pr. rettgeri	410	4·8	3,800	—	—

—, Destruction too slow to be measured accurately (that is, < 10).

substrates was investigated, using cell-free bacterial extracts, by the use of the spectrophotometric assay. From the progress curves obtained, Michaelis constants were calculated where possible; results are shown in Table 2. Cephaloridine has a lower affinity, as can be seen, for the penicillinases from the *K. aerogenes* and *E. coli* strains than have either benzylpenicillin or ampicillin; thus, at concentrations that are obtained in the blood[1] during therapy (approximately 20 μM), cephaloridine will be at least 17 times as stable as ampicillin to *K. aerogenes* penicillinase. In the case of the *A. cloacae* enzyme, the affinity of cephaloridine is some 8 times as high as that of ampicillin. Thus, by calculation, towards the penicillinase of this organism cephaloridine will be 3·5 times as labile as ampicillin, at concentrations attained therapeutically; this should be contrasted with the situation at 5 mM (Table 1), when cephaloridine is less labile than ampicillin.

Ease of entry into intact bacteria. Most penicillinase-producing Gram-negative bacteria studied in this laboratory[11] possess accessibility barriers against penicillins; the extent of the barrier for any one substrate is expressed as the 'permeability factor'[14] (which is the fold increase in hydrolytic activity toward a substrate subsequent to disruption of an intact bacterial suspension). Permeability factors have been determined for benzylpenicillin, ampicillin, 6-aminopenicillanic acid and cephaloridine for 18 strains; values are tabulated in Table 3. The *Proteus* strains have been omitted because the extent of the permeability barrier depends on the nature and the concentration of the inducing agent[13]. From the results it is quite clear that cephaloridine can enter bacterial cells of every strain tested with complete freedom, whereas these same cells possess a marked accessibility barrier to benzylpenicillin and ampicillin. 6-aminopenicillanic acid, although obtaining considerably easier access than the latter two compounds, does not have complete freedom of entry in all strains. A consequence of this free access is that, whereas the crude penicillinase of *A. cloacae* 53 hydrolyses cephaloridine at 61 per cent of the rate of ampicillin (to which the intact cells have a permeability factor of 8), cephaloridine was found to be hydrolysed by intact cells about 5 times as rapidly as ampicillin. There does not seem to be an adequate explanation, in terms of molecular size, charge or the like, for this property of cephaloridine. One explanation which could be put forward to account for this phenomenon is that cephaloridine affects the cells so that the penicillinase becomes extracellular and thus there is no longer any barrier between enzyme and substrate. However, this was disproved by the findings in the following experiment.

Table 2. Affinity of Cell-free Penicillinase Preparations for Three Substrates

Michaelis constants expressed as μM

Bacterial strain		Benzylpenicillin	Ampicillin	Cephaloridine
A. cloacae	53	⩽8	541	69
K. aerogenes	366	⩽68	⩽143	1,330
K. aerogenes	373	⩽68	⩽143	1,200
E. coli	419	⩽68	*	276

* No appreciable hydrolysis was detected at any substrate concentration.

Table 3. Permeability Factors for 4 Drugs and 18 Bacterial Strains, measured in 25 mM Phosphate Buffer *p*H 7·4

Strain of bacteria		Benzyl-penicillin	Ampicillin	6-Aminopeni-cillanic acid	Cephaloridine
A. cloacae	15	1	1	1	1
,, ,,	53	18	8	1	1
E. coli	214*T*	10	—	—	1
,, ,,	419	4	—	—	1
,, ,,	*K*12	1	—	—	1
,, ,,	*TEM*	2	—	—	1
K. aerogenes	1	6	7	1	1
,, ,,	43	11	13	2	1
,, ,,	83	2	2	2	1
,, ,,	366	11	14	2	1
,, ,,	370	8	8	1	1
,, ,,	373	9	5	2	1
,, ,,	402	3	2	3	1
,, ,,	407	3	2	1	1
,, ,,	414	2	2	2	1
,, ,,	415	4	3	1	1
,, ,,	418	4	4	1	1
K. ozaenae	61	3	3	1	1

Table 4. Inhibition of Benzylpenicillin and Cephaloridine Hydrolysis by Cloxacillin, Methicillin and Quinacillin

The concentration of inhibitor required to cause 50 per cent inhibition (I_{50}) is expressed as μM (initial substrate concentration was 2·7 mM)

Penicillinase prep.		Substrate	I_{50} values Cloxacillin	Methicillin	Quinacillin
K. aerogenes	366	Benzylpenicillin	431*	10*	70†
		Cephaloridine	80	0·32	12·4
K. aerogenes	373	Benzylpenicillin	711*	7*	161†
		Cephaloridine	90·5	0·4	25·4
E. coli	419	Benzylpenicillin	0·8	52·4	> 1,090
		Cephaloridine	0·1	4·8	392

* Taken from ref. 15.
† Taken from ref. 14.

Table 5. Minimum Inhibitory Concentrations (μg/ml.) of Cephaloridine and Ampicillin for 16 Strains of Penicillinase-producing Gram-negative Bacteria

Inoculum		Cephaloridine 10^7	Cephaloridine 10	Ampicillin 10^7	Ampicillin 10
Pr. morgani	*G*	2,500	313	500	125
,, ,,	185	1,250	39	250	31
Pr. rettgeri	410	2,500	5	2,000	12
K. aerogenes	1	125	31	2,000	31
,, ,,	43	125	16	1,000	31
,, ,,	370	125	4	2,000	16
,, ,,	402	25	3	1,000	31
,, ,,	407	32	2	1,000	16
,, ,,	414	31	4	1,000	16
,, ,,	415	25	3	200	13
,, ,,	418	12	2	1,000	25
A. cloacae	15	100	10	125	10
,, ,,	53	8,000	500	20,000	3,200
E. coli	214*T*	200	25	250	250
,, ,,	419	2,000	16	2,000	2,000
,, ,,	*K*12	16	2	8	2

Whole-cell suspensions were assayed against cephaloridine (initial concentration 5 mM); they were then spun down, re-suspended in fresh buffer and re-assayed against cephaloridine. It was found, allowing for small losses due to lysis and the centrifugation and re-suspending operations, that the pre-incubation with cephaloridine had not resulted in any loss of enzyme from the intact cells into the supernatant fluid.

Inhibition of pencillinase activity. The hydrolysis of benzylpenicillin by penicillinases from Gram-negative bacteria is inhibited by methicillin, certain isoxazolyl penicillins and quinacillin[14,15]. I_{50} values[15] were determined, using cephaloridine (2·7 mM) as substrate, for methicillin, cloxacillin and quinacillin; results are shown in Table 4 (the I_{50} values obtained previously[14,15] using benzylpenicillin as substrate for *K. aerogenes* 366 and 373 enzymes are included for comparison); it is clear that cephaloridine hydrolysis by these penicillinases is more susceptible to inhibition than is benzylpenicillin hydrolysis. Inhibition of cephaloridine hydrolysis by methicillin appears to be competitive, as increasing the substrate concentration with a given amount of inhibitor decreases the amount of inhibition.

Antibacterial activity against penicillinase-producing Gram-negative bacteria. Minimum inhibitory concentrations were determined for cephaloridine, using the serial doubling dilution technique; two sizes of inoculum were used, about 10^7 and 10 organisms per tube, and incubation was carried out for 16 h. Results are shown in Table 5. It can be seen that cephaloridine is approximately ten times as effective as ampicillin against the penicillinase-producing ampicillin-resistant *K. aerogenes* strains; against the other strains there seems to be little to choose between cephaloridine and ampicillin as regards antibacterial activity. In most cases there was a marked inoculum size effect[16].

It is generally accepted that a direct causal relationship exists between penicillinase activity and resistance to hydrolysable penicillins. Cephaloridine was destroyed more rapidly than ampicillin by intact cells from the majority of the strains studied here and hence it would be expected that cephaloridine would be a less effective antibacterial agent than ampicillin. However, with the exception of the *Proteus* strains, the reverse was found to be true with the strains examined in this report. This apparently paradoxical situation can be explained by the discovery made here that the entry of cephaloridine into these Gram-negative bacteria is not hindered by a cellular

accessibility barrier, whereas entry of all the penicillins is so hindered[11,12].

With eight strains of penicillinase-producing ampicillin-resistant *K. aerogenes* cephaloridine was found to be more effective than ampicillin, in terms of minimum inhibitory concentrations, against both large and small inocula. Even so, therapeutic administration of cephaloridine may not give rise to blood levels[1] sufficiently high to have an antibacterial action *in vivo* with these strains, but the levels attainable in urine[1] suggest that cephaloridine may have a place in the treatment of urinary tract infections caused by similar organisms. However, the *in vivo* performance of a drug cannot be predicted solely in terms of its effectiveness in *in vitro* sensitivity tests. Other factors, for example, absorption, excretion and possible synergism with penicillinase-resistant β-lactam antibiotics, must be considered before this antibiotic can be recommended for use in infections caused by penicillinase-producing Gram-negative bacteria.

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THE IRON DEXTRAN COMPLEX

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PHARMACEUTICAL preparations ('Imferon' and 'Imposil', Fisons Pharmaceuticals, Ltd. (Benger Laboratories)) containing iron and dextran are used for the treatment of iron-deficiency anaemia in man and farm animals. They are prepared by neutralizing ferric chloride in the presence of an alkali-modified dextran and heating to form a stable clear brown solution. The nature of the linkage between the iron and the dextran and the molecular weight of the product are not known. The following techniques seemed likely to throw light on the nature of the iron dextran preparation: (1) gel-filtration on 'Sephadex'; (2) ultrafiltration; (3) separation in the ultracentrifuge in comparison with coloured proteins of known molecular weight.

Separation on 'Sephadex' columns. A column of 'Sephadex *G*200' (2·4 cm × 30 cm) using 0·9 per cent sodium chloride for elution was available and had previously been calibrated with dextran fractions of known molecular weight distribution (Pharmacia, Ltd.) and with serum proteins. The reproducibility of chromatography had been established using a sample of dextran labelled with iodine-125 (ref. 1); on successive runs the size of each fraction was reproducible within ±1 per cent of the whole. In an initial experiment 'Imferon' (1 ml.) was mixed with 0·1 ml. of 10 per cent iodine-125 dextran and diluted to 5 ml. with saline. A sample of 1 ml. of this diluted solution was applied to the column and eluted with saline into 5-ml. fractions. Part of the iron was adsorbed on the 'Sephadex' making the whole column brown, the remaining iron being eluted. The adsorbed iron could not be eluted with saline and was finally recovered by elution with N sulphuric acid.

The radioactivity, the brown colour (430 mμ) of iron and the dextran concentration of the fractions are shown in Fig. 1. The radioactivity measurements showed that the chromatography was comparable with previous calibrations. From the position of the iron peak one might infer that the molecular weight of the iron dextran complex was less than that of the labelled dextran which had $\bar{M}_w$ about 110,000. The peak lies between the positions of peaks previously found for dextrans of $\bar{M}_w$ 75,000 and $\bar{M}_w$ 40,000 and is closer to the lower value. The dextran peak is adjacent to the peak due to inorganic iodide ions and is to the low molecular weight side of a peak previously obtained with a dextran fraction of $\bar{M}_w$ 9,400; its position is thus consistent with a molecular weight of about 5,000. However, the shape of this peak

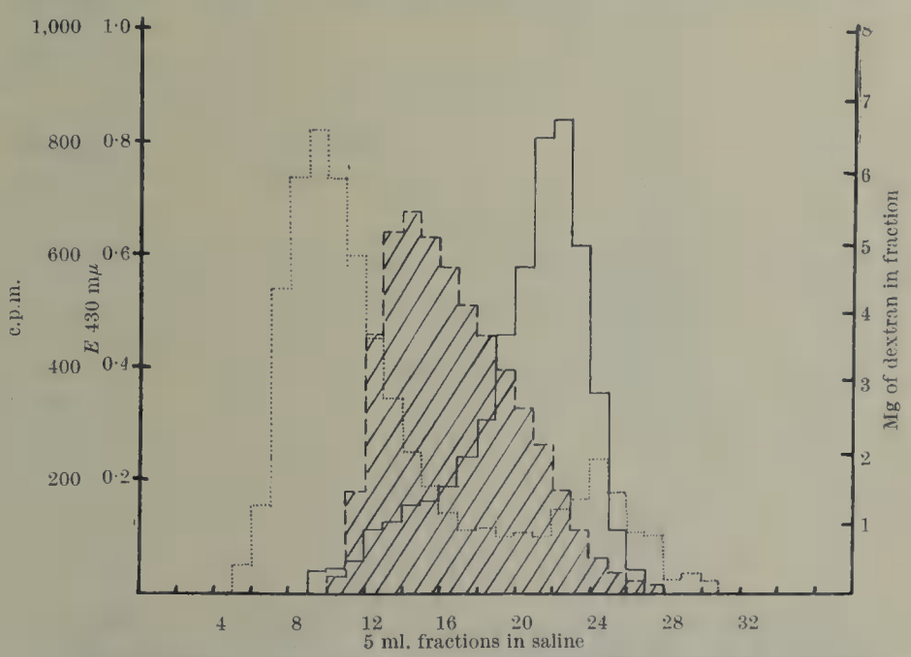

Fig. 1. Gel-filtration of 'Imferon' on a column of 'Sephadex *G*200' in 0·9 per cent sodium chloride solution. The dotted line shows the distribution of radioactivity from ^{125}I dextran of $\bar{M}_w$ 110,000 used as a reference substance. The dashed line and shaded area denote the distribution of iron and the solid line denotes the distribution of dextran from the 'Imferon'

is worth considering. Dextrans of $\bar{M}_w$ 40,000 and below give a symmetrical peak on columns of 'Sephadex *G*200'. Dextrans of higher molecular weight give an unsymmetrical peak, sharpened on the high molecular weight side, that is, the tail toward the low molecular weight side. The dextran peak in Fig. 1 has a tail toward the high molecular side, suggesting that the iron is carrying some of the dextran through the column ahead of most of the dextran. This is an indication of the existence of a linkage between the iron and the dextran existing under the physiological conditions of isotonic saline and a measured *p*H of 6·8. However, adding up the material in the fractions, 101 per cent of the radioactivity, 97·7 per cent of the dextran but only 32 per cent of the iron were recovered. Evidently 68 per cent of the iron had been separated from dextran under these mild conditions, possibly through competition for the iron between the dextran and 'Sephadex', which is a cross-linked and insoluble form of dextran.

This initial experiment suggested that 'Sephadex *G*75' might separate the iron–dextran complex from free dextran. Molar sodium sulphate solution was chosen in an attempt to minimize adsorption of iron on to 'Sephadex'.

A sample of 0·5 ml. of 1 in 5 dilution of 'Imferon' was applied to a column of 'Sephadex *G*75' (1·4 cm × 48·5 cm) and eluted into 2 ml. fractions with M sodium sulphate. The separation of the iron complex from free dextran is shown in Fig. 2. Recovery of the iron was 87·4 per cent and of dextran was 95·5 per cent. Of the recovered dextran 61·4 per cent was free and the remainder was associated with iron. The iron–dextran ratio of fractions 9, 10 and 11 was 0·536, 0·533 and 0·470 respectively, that is, about two parts of dextran to one part of iron.

It was found that under the same conditions the dextran fraction used in the preparation of 'Imferon' was eluted in the same volume as the second dextran peak of Fig. 2. This would indicate that the second peak was either dextran liberated by breakdown of the complex, free dextran which was present in 'Imferon' but not complexed with iron or a combination of these.

Later experiments using a 'Sephadex *G*75' column showed that between 75–85 per cent of the iron could be recovered in the iron–dextran peak by elution with 0·9 per cent sodium chloride. These suggest that it may not have been an effect of ionic strength which was governing the adsorption of the iron on to 'Sephadex', but the length of time that the 'Imferon' was in contact

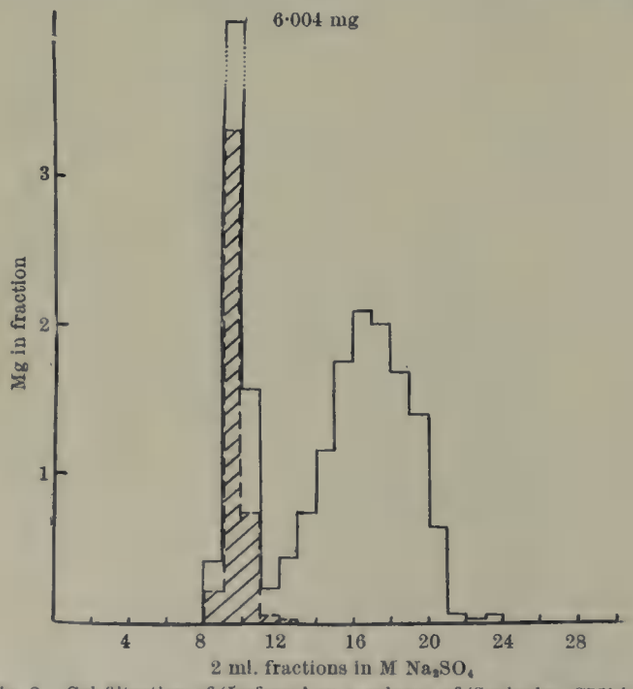

Fig. 2. Gel filtration of 'Imferon' on a column of 'Sephadex *G*75' in M sodium sulphate solution. The hatched area denotes the distribution of iron and the solid line denotes the distribution of dextran. —, Mg dextran in fraction; – – –, mg iron in fraction

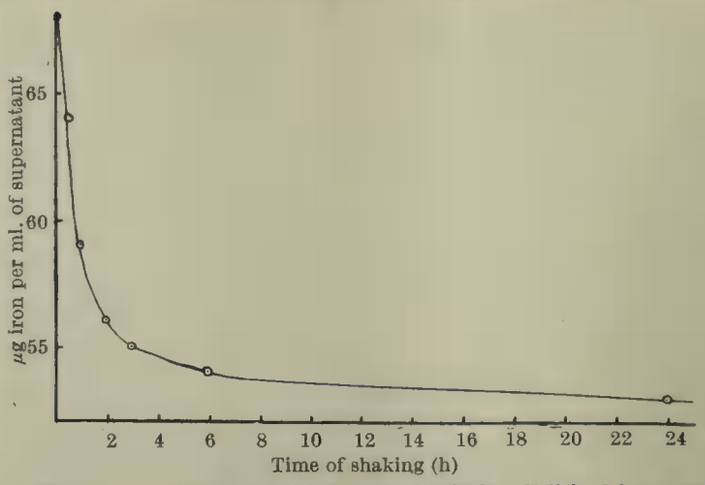

Fig. 3. The adsorption of iron on to 'Sephadex *G*75' in 0·9 per cent sodium chloride solution

with the dextran gel. This was confirmed by examining the effect of flow-rate on the adsorption of iron on a 'Sephadex *G*75' column. By elution at a flow-rate of 100 ml./h 70 per cent of the iron was recovered, whereas at 5 ml./h only 20 per cent of the iron was recovered.

There were no differences in the relative peak positions for the two fractionations but the amount of dextran associated with the iron in the first peak varied according to the flow-rate so that the iron to dextran ratio was 0·33 : 1 with a short elution time and 0·5 : 1 with a long elution time.

In order to examine further the effect of time on the adsorption of iron on to 'Sephadex' a 1-ml. aliquot of a 1 in 20 dilution of 'Imferon' was added to 1 gm of 'Sephadex *G*75' in 50 ml. of 0·9 per cent sodium chloride. The mixture was shaken for 5 min and a 2-ml. aliquot removed, centrifuged and the iron content estimated on the clear supernatant using bathophenanthroline reagent[2]. The mixture of 'Imferon' and 'Sephadex *G*75' was shaken for 24 h, 2-ml. samples being removed at intervals, centrifuged and the iron contents of the supernatants estimated. Fig. 3 shows the effect of plotting iron content of the supernatant against the time the 'Imferon' was in contact with 'Sephadex'. By 24 h, when an equilibrium appeared to have been established, 22 per cent of the added iron had been adsorbed by the 'Sephadex'. During the first 2–3 h there was a very rapid fall in the iron content of the supernatant while during the next 20 h the rate of adsorption of the iron was very much slower.

When the amount of added 'Imferon' was reduced to one-tenth of that in the previous experiment a similar result was obtained with 30 per cent of the iron being bound to the 'Sephadex'. Using a polyacrilamide gel in place of 'Sephadex' gave a similar result.

Ultrafiltration. The 'Sephadex' gel fractionation suggested that 'Imferon' might contain uncombined dextran and it was thought that this could be more easily separated using ultrafiltration.

'Imferon' was not retained by a membrane with porosity of 10 mμ, but cellulose ('Visking') film retained the iron–dextran complex while allowing uncombined dextran to pass through extremely slowly. In order to increase the pore size of the filter the membrane was treated with 60 per cent zinc chloride solution for 10 min. Membranes treated in such a way completely retained bovine haemoglobin, which has a diameter of 2·7 mμ. A solution of 10 ml. of 'Imferon' diluted to 40 ml. with water was subjected to filtration through such a membrane at 80 lb./in.^{2} pressure. At intervals the volume of the 'Imferon' solution was restored by adding quantities of water equal to the volume of fluid which had passed through the filter. After a period of three weeks a total of 160 ml. had filtered. Table 1 shows the analysis of the solutions.

Zone ultracentrifugation in a density gradient. Density gradient tubes were prepared[3] containing carbon tetra-

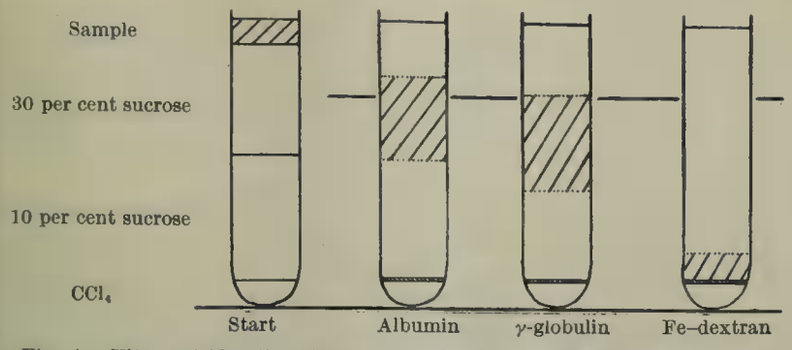

Fig. 4. Ultracentrifugation, in a sucrose density gradient, of 'Imferon' and for comparison serum proteins coupled with 'Procion' dyes

chloride (0·3 ml.), which has a density of 1·59, 30 per cent sucrose solution (2·3 ml.) of density 1·26 and 10 per cent sucrose (2·3 ml.) of density 1·07. The sample (0·2 ml.), having a density of less than 1·07, was carefully layered on top and the tubes were centrifuged at 35,000 r.p.m. for 16 h. Samples used were a 1 in 20 dilution of 'Imferon', a 4 per cent solution of albumin coupled with 'Procion' red dye (Imperial Chemical Industries, Ltd.) and a 4 per cent solution of γ-globulin coupled with 'Procion' blue dye (Imperial Chemical Industries, Ltd.). After centrifuging, the tubes presented the appearance shown in Fig. 4. Traces of insoluble protein had collected at the carbon tetrachloride interface. The iron colour was close to and at the interface, indicating a much higher molecular weight than albumin 69,000 or γ-globulin 156,000.

Discussion

In the initial 'Sephadex' gel filtration experiment the position of the eluted iron peak lay between the positions previously found for dextrans of $\bar{M}_w$ 75,000 and 40,000.

Table 1. Analysis of Solutions obtained by Ultrafiltration of 'Imferon'

	Iron concentration	Dextran concentration	Iron : dextran ratio
Original 'Imferon' solution	1·22%	5·42%	0·224
Final 'Imferon' solution	2·8%	6·7%	0·418
First 10 ml. filtrate	50 p.p.m.	2·3%	—
Final 10 ml. filtrate	1 p.p.m.	0·14%	—

However, it seems very likely that the iron peak was retarded in its progress through the column by adsorption so that its position did not truly indicate molecular size. Adsorption of iron in this way would increase the proportion of free dextran detected.

The ultracentrifugation experiment showed that the iron–dextran complex has a molecular or particle size much greater than that of γ-globulin. Muir and Golberg[4] showed by electron microscopy the presence of electron-dense particles 2–3 mμ in diameter, and the particulate nature of the complex was confirmed by an electron micrograph of one of the fractions (fraction 10, Fig. 2) which showed particles approximately 3–4 mμ in diameter.

The ultrafiltration experiments showed conclusively that a large proportion of uncombined dextran (42·2 per cent) was present. By this means it was possible to remove the latter, leaving what was presumably a solution containing only iron–dextran complex. The ratio of iron to dextran in this solution was 0·418 : 1, which is slightly higher than that calculated for one iron atom per anhydroglucose unit, in which the ratio is 0·346 : 1. The ratio corresponds with 2·4 parts of dextran to 1 part of iron.

The molecular weight of the dextran from which the preparation is made is little more than 5,000. In order to have a molecular weight greatly exceeding 150,000 and a particle size of 2–3 mμ there must be considerable cross-linking or association of molecules to form the complex. Further investigations are being carried out on this aspect.

Electron micrographs of fraction 10 were kindly prepared and interpreted by Dr. A. T. Charles, Medical Research Council Unit for Research on the Experimental Pathology of the Skin, University of Birmingham.

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PENICILLINASE SYNTHESIS CONTROLLED BY INFECTIOUS *R* FACTORS IN ENTEROBACTERIACEAE

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RESISTANCE to antibacterial drugs may be transferred from one bacterium to another among the Enterobacteriaceae by direct cell-to-cell contact (conjugation). This transferable resistance was discovered in Japan[1–3] and later reported in Britain[4] and in Germany[5]. The infectious agents which mediate the transfer are known as resistance factors (*R* factors) and have been shown to be extra-chromosomal genetic elements, or plasmids, consisting of deoxyribonucleic acid[3,6]. *R* factors are transferred by conjugation between cells of many genera—all the Enterobacteriaceae[7] as well as other Gram-negative bacilli such as *Vibrio*[8], *Serratia*[9] and *Pasteurella*[10]. The resistance conferred by the *R* factors first described was against the four drugs streptomycin, tetracycline, chloramphenicol and sulphonamides, usually to all four together, but sometimes against three, two or one of them. Lebek[5] described an *R* factor which conferred resistance to neomycin and kanamycin, as well as to the original four drugs.

That genetic information for resistance to penicillins, including ampicillin, could be carried on *R* factors was found independently in Britain[11], in Greece (Kontomichalou, unpublished data) and in Switzerland (Lebek, personal communication). Preliminary experiments[11] suggested that infectious resistance to penicillins was dependent on the production of a penicillin-destroying enzyme. We now report that this enzyme is penicillinase (*EC* 3.5.2.6), since on incubation with benzylpenicillin it liberates penicilloic acid. We therefore present evidence that the genetic information for the biosynthesis of penicillinase is carried on the *R* factor.

R factors and host bacteria. The *R* factors we have studied are listed in Table 1. The host bacteria in which penicillin resistance was studied were *Escherichia coli K*12, *E. coli* strain *TEM* the *R* factor of which had been eliminated by acriflavine treatment[12], and *Salmonella typhi* strain 152, a drug-sensitive strain isolated in Greece in 1962.

Table 1

R factor	Species in which isolated	Year of isolation	Place of isolation	Pattern of infective resistance
R_{TEM}	*Escherichia coli*, strain *TEM*	1963	Athens	*A S*
R_{1818}	*Salmonella typhimurium* (ref. 11) phage type 1*a*	1962	Brighton	*A S T Su*
R_{7268}	*Salmonella paratyphi* B	1963	London	*A C Su K*

Species resistant to: *A*, ampicillin; *C*, chloramphenicol; *S*, streptomycin; *Su*, sulphonamides; *T*, tetracycline; *K*, kanamycin.

The *R* factors were transferred from their original hosts to the bacterial strains used experimentally by growing resistant and sensitive cultures together in broth, and plating on medium selective for the recipient organisms, but incorporating ampicillin. For example, in transferring resistance from *Salmonella* to *E. coli* the mixtures were plated on minimal agar with lactose as sole *C* source and with ampicillin, 25 μg/ml.

Stability of R factors in their bacterial hosts. Cultures carrying *R* factors contain a variable proportion of drug-sensitive cells from which the factor has been entirely lost[1,2]. The rate of loss varies greatly between different *R* factors and between different hosts. The rate of loss of all the 3 *R* factors we describe here was 1 : 1,000 cell divisions or less, in the original hosts as well as in *E. coli K*12, *E. coli TEM* and *S. typhi* 152.

'Training' to ampicillin resistance. The sensitive host bacteria, *E. coli K*12 and *E. coli TEM*, were 'trained' to become resistant to ampicillin by passage on nutrient agar gradient plates containing increasing concentrations of ampicillin. These laboratory-trained resistant cultures were compared with *R* factor infected cultures in the experiments described later (see Table 2).

Extraction of enzymes. No penicillinase was found in supernatant fluid from our cultures carrying *R* factors. Highest yields of enzyme were from suspensions broken ultrasonically with a Mullard drill. Fast centrifugation (105,000*g* for 2 h) of the ultrasonicates left all activity in the supernatant. Methods of extracting enzyme without disrupting the cells, such as by toluene treatment, ethylenediamine tetraacetic acid (EDTA) at *p*H 8 (ref. 13) or EDTA + 20 per cent (w/v) sucrose at *p*H 8 (ref. 14), liberated up to 35 per cent of the penicillinase activity found in ultrasonicated preparations. Extraction of the enzyme by these methods, which leave the cell membrane intact, indicates that, like some other degradative enzymes in *E. coli*[14], these penicillinases are 'periplasmic' enzymes.

Penicillinase assays. The cultures with and without *R* factors (*R*+ and *R*−) were assayed iodometrically for hydrolysis of benzylpenicillin, ampicillin and cephaloridine, the latter two drugs being chosen because of their clinical importance in treatment of infection caused by Gram-negative bacilli. 6-Amino penicillanic acid (6-APA) was also used, because it had been found[15] to be a highly effective antibiotic against at least one strain of penicillinase-producing Gram-negative bacilli.

Bacteria from exponential phase-aerated broth cultures were collected by centrifugation and the bacteria resuspended in distilled water, the volume being chosen according to the expected enzyme activity of the preparation. Assays were made using these suspensions untreated and after ultrasonic disruption. The method of assay was that of Perret[16] as modified by Novick[17]. Hydrolysis of cephaloridine was measured similarly to that of benzylpenicillin, ampicillin and 6-APA, except that the amount destroyed was calculated on a basis of 1 mole hydrolysed cephaloridine being equivalent to 4 equivalents of iodine[18]. The enzyme activities of the preparations were expressed in the units defined by Pollock and Torriani[19]: 1 unit is equivalent to 1 μmole penicillin hydrolysed/h at 30° and *p*H 7·0.

Levels of resistance to penicillin, ampicillin, cephaloridine and 6-APA. The presence of *R* factors in the cultures was recognized by their greatly increased resistance to ampicillin. We measured the levels of resistance of our cultures against the four drugs used as substrates in the enzyme assays. Aerated broth cultures in the exponential phase were diluted to contain approximately 10^7 organisms/ml. and 10^3 organisms/ml. These suspensions were dropped from calibrated pipettes on nutrient agar plates incorporating serial dilutions of the drugs under test. The heavier suspension gave confluent growth on drug-free control plates; the light suspension yielded isolated colonies. The minimal inhibitory concentration (M.I.C.) recorded was the lowest which prevented visible growth.

The M.I.C.s and the results of enzyme assays are summarized in Table 2.

From Table 2 the following observations may be made:

(1) The host *E. coli* cultures, both *K*12 and *TEM*, without *R* factors, were drug-sensitive and had very low, but just measurable, hydrolytic activity against benzylpenicillin and cephaloridine, but none against ampicillin or 6-APA. *S. typhi* 152 was considerably more sensitive to benzylpenicillin and ampicillin than our *E. coli* strains. No penicillinase was detected in *S. typhi* 152 without *R* factors, but it may, nevertheless, possess very low activities since we did not assay highly concentrated suspensions of

Table 2. Levels of Resistance and Penicillinase Activity conferred by *R* Factors

R factor	Host bacteria	Minimal inhibitory concentration (μg/ml. medium)					Enzyme activity (units/h/mg dry weight)				
		Inoculum (No. bacteria/ml.)	Benzyl-penicillin	Ampicillin	Cepha-loridine	6-APA		Benzyl-penicillin	Ampi-cillin	Cepha-loridine	6-APA
	*E. coli K*12	10^3	15	1	1	15	Intact	—	—	0·2	—
		10^7	30	2	2	30	Broken	0·1	—	0·3	—
	E. coli TEM 'cured'	10^3	15	2	1	15	Intact	—	—	0·1	—
		10^7	30	2	2	30	Broken	—	—	0·2	—
	S. typhi 152	10^3	2	< 0·5	1	15	Intact	—	—	—	—
		10^7	4	< 0·5	1	30	Broken	—	—	—	—
	*K*12 ampicillin 'trained'	10^3	1,000	125	15	15	Intact	0·05	—	0·2	—
		10^7	1,000	250	15	30	Broken	0·10	—	0·3	—
	TEM 'cured' ampicillin 'trained'	10^3	500	125	30	15	Intact	0·05	—	0·3	—
		10^7	500	125	30	30	Broken	0·05	—	0·3	—
TEM	*K*12	10^3	1,000	1,000	6	2,000	Intact	1·7	6	170	98
		10^7	4,000	4,000	60	4,000	Broken	125	180	210	175
	TEM	10^3	1,000	1,000	6	2,000	Intact	1·2	3·2	106	51
		10^7	> 4,000	4,000	60	4,000	Broken	80	120	140	106
	S. typhi 152	10^3	1,000	1,000	6	2,000	Intact	14	28	215	290
		10^7	4,000	4,000	60	4,000	Broken	200	320	330	370
1818	*K*12	10^3	30	30	2	30	Intact	0·5	0·5	1·4	1
		10^7	250	250	4	60	Broken	2·4	2·2	1·9	1·6
	TEM 'cured'	10^3	30	30	2	30	Intact	0·4	0·8	1·8	1·4
		10^7	200	200	4	60	Broken	2·5	2·8	2·4	1·7
7268	*K*12	10^3	250	250	4	1,000	Intact	0·5	2	20	12
		10^7	1,000	1,000	8	2,000	Broken	22	22	32	19
	TEM 'cured'	10^3	250	250	4	1,000	Intact	0·3	2	18	13
		10^7	1,000	1,000	8	2,000	Broken	18	17	31	22

—, No detectable activity. Lowest detectable activity: in *E. coli*, 0·05 units/h/mg dry wt; in *S. typhi*, 0·5 units/h/mg dry wt.

this organism, as we did for the non-pathogenic ones. 'Training' of our *E. coli* cultures on ampicillin did not lead to their developing appreciably higher hydrolytic activity against any of the four substrates, and there was still no detectable hydrolysis of ampicillin or 6-APA.

(2) Acquisition of *R* factors by the cultures resulted in greatly increased resistance to benzylpenicillin, ampicillin and 6-APA, and greatly increased hydrolytic activity against all four drugs tested. The levels of drug-resistance attained by the *R*+ bacteria were different for each of the three *R* factors studied. The levels of resistance were clearly related to penicillinase activity, the most resistant having highest penicillinase activity and the least resistant, lowest.

(3) Different host bacteria carrying the same *R* factor showed rather similar penicillinase activities. With all three factors, *E. coli K*12 showed slightly but consistently higher activity than *E. coli TEM* and, where tested, *S. typhi* 152 showed even higher activity, but these differences were not accompanied by differences in the levels of drug-resistance.

(4) Whole *R*+ cell suspensions, assayed for hydrolysis of penicillin and ampicillin, gave low levels of activity, indicating that the penicillinase was inaccessible to these substrates unless liberated by ultrasonic disruption (or any of the other methods already discussed). For cephaloridine and 6-APA, on the contrary, the difference in the rate of hydrolysis by whole cell suspensions and by ultrasonicated ones was small, indicating that for these substrates the permeability barrier excluding penicillin and ampicillin was not effective. Table 2 also shows that the *R*+ cultures, although their hydrolytic activity against cephaloridine is raised to the same degree as that against benzylpenicillin and ampicillin, have not acquired nearly such a high degree of resistance to cephaloridine as to the penicillins.

(5) The *R*− cultures were more sensitive to ampicillin than to benzylpenicillin but the *R*+ cultures were equally resistant to both.

Substrate profiles. The penicillinase activity of some of our cultures carrying *R* factors was assayed against a wider range of substrates than shown in Table 2 (see Table 3). The penicillinase produced by three different hosts infected by one *R* factor (R_{TEM}) all showed similar substrate profiles. But when the penicillinases from one host (*E. coli K*12), infected with three different *R* factors, were compared they showed interesting differences in substrate profiles. Penicillinase from *E. coli K*12 with factor R_{7268} had a similar substrate profile to that with R_{TEM}, but with R_{1818} the profile was quite different; with R_{1818} cloxacillin was hydrolysed as rapidly as benzylpenicillin and phenoxyethyl- and phenoxypropyl-penicillin considerably more rapidly still.

Induction. Tests for inducibility of penicillinase have so far been limited to the examination of *E. coli K*12 carrying factor R_{1818}. When methicillin was added to a growing culture no increase in penicillinase activity was observed.

Discussion. The synthesis of penicillinase by our cultures carrying *R* factors appears to be under the direct genetic control of the *R* factors. It might be argued that, since the host cultures without *R* factors themselves produce some penicillinase, the *R* factors act only to remove a repressor mechanism and so increase penicillinase activity; extracts of our *R*+ cultures differ, however, from those of our *R*− cultures not only in their greatly increased penicillinase activity, but also in having high activity against ampicillin and 6-APA. We believe, therefore, that the *R*+ cultures synthesize penicillinases which are not present in the *R*− cultures. Penicillinases from our *R*− *E. coli* strains resemble in their substrate range those of *E. coli* strains described by Smith and Hamilton-Miller[20,24], while the same *E. coli* strains, carrying *R* factors, synthesize penicillinases with very different substrate profiles.

Table 3. Relative Rates of Hydrolysis of Various Substrates by Ultrasonically Disrupted Preparation of *R*+ Cultures

R factor	R_{TEM}	R_{TEM}	R_{TEM}	R_{1818}	R_{7268}
Host bacteria	*S. typhi* 152	*E. coli TEM*	*E. coli K*12	*E. coli K*12	*E. coli K*12
Substrate					
Benzylpenicillin	100	100	100	100	100
Ampicillin	160	150	145	91	100
6-APA	165	142	132	73	86
Methicillin	< 0·1	0·6	< 0·1	21	< 0·1
Cloxacillin	1·5	2·3	1·8	126	1·5
Cephaloridine	180	175	168	86	145
Cephalothin	16	19	15	46	17
Phenoxymethylpenicillin	61	57	69	92	50
Phenoxyethylpenicillin			24	180	24
Phenoxypropylpenicillin			8·5	240	7

The penicillinases synthesized by *E. coli K*12 carrying factors R_{TEM} and R_{7268} resemble one another in their substrate profiles, although not in their levels of activity. Their substrate profiles resemble those of extracts of *Aerobacter* (*Klebsiella*) *aerogenes* described by Smith and Hamilton-Miller[20]. We do not yet know whether each enzyme is specific to its *R* factor or whether these two factors both determine the synthesis of the same enzyme, each at a characteristic rate. The penicillinase of factor R_{1818} differs markedly from the others, and from all penicillinases so far described, in hydrolysing cloxacillin as rapidly as benzylpenicillin and in hydrolysing phenoxymethyl and phenoxypropyl penicillins considerably faster still.

The parallelism between the levels of drug-resistance and the penicillinase activities conferred by the different *R* factors strongly suggests that the resistance was the direct result of the presence of penicillinase in the cells. The increase of drug-resistance with drug-hydrolysis was much less with cephaloridine than with the penicillins; thus the penetration of a drug into the cell may increase the antibacterial effectiveness of the drug, as indicated by Hamilton-Miller[15]; however, in spite of the permeability of the cells to 6-APA, the latter was not a highly effective antibiotic against our strains.

It is especially interesting that penicillinase biosynthesis can be controlled by infective extrachromosomal elements in the Enterobacteriaceae, since it is known that penicillinase in staphylococci is controlled by comparable, though non-infective, plasmids[21–23].

We thank Mrs. Roma Pride for her assistance and Dr. M. H. Richmond for his advice.

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DEOXYRIBONUCLEIC ACID SYNTHESIS IN CULTURED HUMAN CELLS AND ITS BEARING ON THE CONCEPTS OF ENDOREDUPLICATION AND POLYPLOIDY

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THE synthesis of deoxyribonucleic acid (DNA) is a prerequisite for mitosis, but it is now known that the two processes are essentially separate[1,2]. If DNA synthesis occurs in excess of requirements for the production of new cells, some of the existing cells will become polyploid. We wish to present some data showing that this happens in human fibroblast-like cells grown in tissue culture.

The cells originated from skin biopsies which were cultured according to the technique of Harnden[3]. Culture *C*, which had been growing for five weeks and had been sub-cultured 4 times, had an apparently normal female karyotype, although the cells originated from a male; culture *D*, grown also for 5 weeks and sub-cultured three times, had two *X*-chromosomes and a structural abnormality of a small autosome, which will be described by Crawfurd and Lele[4]. After trypsinization, the cells were allowed to grow on cover slips for about 25 h, when they were fixed in 95 per cent ethanol (0·5 h), hydrolysed in N hydrochloric acid at 60° C (10 min) and stained with Feulgen (1 h). The cover slips were mounted on glass slides. The light absorbed by individual nuclei was measured with an integrating microdensitometer (Barr and Stroud). This instrument was designed by Deeley[5]. The amount of light absorbed by Feulgen-stained nuclei may be regarded as proportional to the amount of DNA present. For diagrammatic representation, the DNA values were converted into logarithms to the base 2, thus separating any value from double its value by one unit. The lengths of the two main nuclear axes were measured with an eyepiece micrometer and the areas were converted into μ^2 with the help of a stage micrometer. From each culture, 1,000 nuclei were measured.

The DNA values, in arbitrary units, of interphase nuclei are shown in Fig. 1. The number of nuclei scored as in mitosis was 87 in culture *C* and 83 in culture *D*. But although the mitotic rates were similar in both cultures, the distributions of DNA values are clearly very different. The approximate number of cells belonging to each DNA class are given in Table 1. Whereas in culture *C* more than two-thirds of the nuclei fall into the diploid class, only about one-third of the nuclei in culture *D* are diploid, and two-thirds are either in the process of DNA synthesis or, having completed this, are polyploid. Although the size of the nuclei varies even within the same DNA class, as is evident from the standard deviations, it will be seen that the mean nuclear areas increase stepwise with increased amounts of DNA. As the nuclei are very flat, their areas may be regarded as proportional to their volumes. The figures in Table 1 show that the increase of about 12 per cent in the average nuclear area of culture *D* compared with culture *C* can be accounted for by a higher incidence of polyploid cells.

It must be emphasized that a high degree of polyploidy is characteristic of the nuclei in interphase only. The incidence of polyploid mitoses was only about 2–3 per cent in both cultures. Wolman *et al.*[6] reported a similar incidence of tetraploid mitoses in tissue cultures during the period of rapid cell division; the incidence of tetraploid mitoses increased with the decline of cell divisions in the culture and following infection with simian virus 40. Polyploid mitoses are common in many tumours, as was shown by Levan[7], and Atkin[8] concluded that for most human tumours the ploidy indicated by DNA values in interphase is in agreement with that obtained from chromosome counts in mitosis. It appears likely, however, that this relationship does not apply to normal dividing tissues, in which the incidence of polyploidy among cells in interphase may be much higher than in mitotic cells.

There can be little doubt that polyploid interphase cells occur also *in vivo*. Thus Swift found DNA values of different multiples in various tissues of the mouse[9] as well as in plants[10]; Swartz[11] reported different DNA

Table 1. Mean Nuclear Areas in Cells with Different DNA Values

DNA value	Culture *C* No. of cells	Culture *C* Mean nuclear area (μ^2)	Culture *C* Standard deviation	Culture *D* No. of cells	Culture *D* Mean nuclear area (μ^2)	Culture *D* Standard deviation
Diploid	659	86	28·1	278	74	31·4
Intermediate	55	100	34·7	206	106	31·3
Tetraploid	194	140	45·5	390	125	38·4
Octoploid	9	263	81·6	39	259	85·9
Total	917	100		913	112	

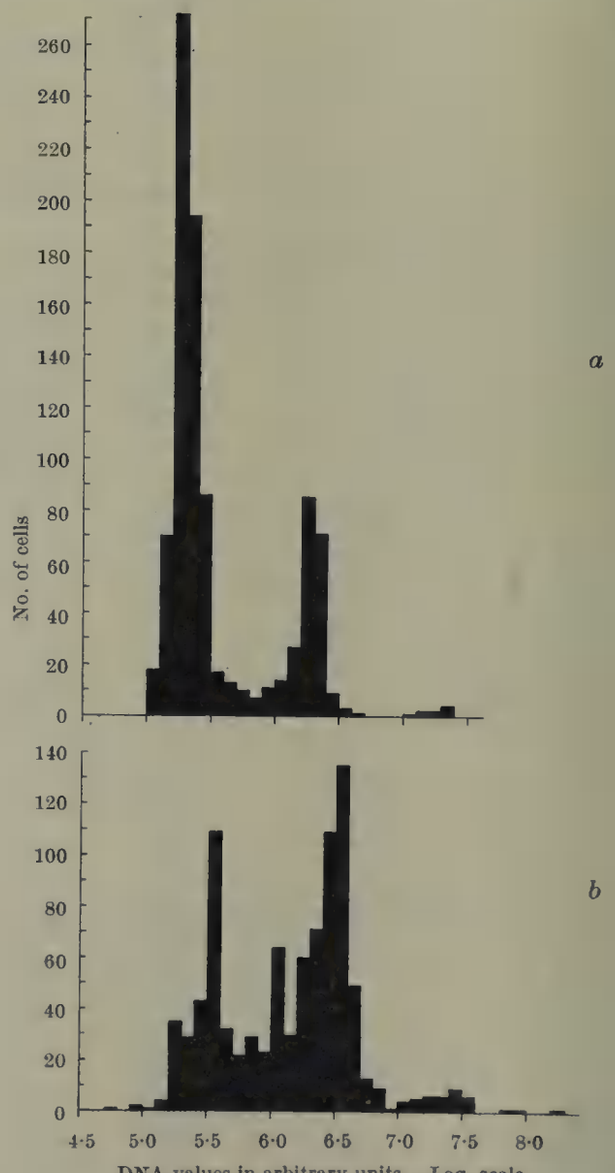


Fig. 1. DNA values in arbitrary units, $\log_2$ scale, of fibroblast-like cells. (*a*) Culture *C*; (*b*) culture *D*

classes in cells of human liver, and Klinger and Schwarzacher[12] in human amnion epithelium and foetal liver.

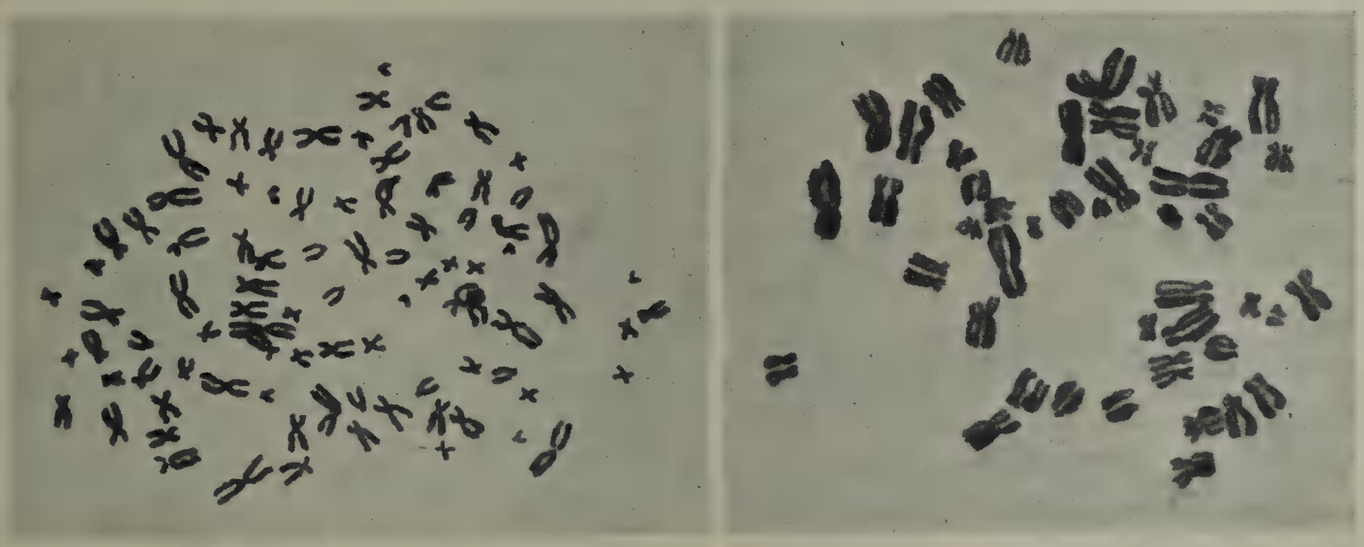
It was shown by Howard and Pelc[13] that in rapidly dividing cells of the bean, *Vicia faba*, the interphase between two mitoses consisted of three stages: (1) a first growth period, $G1$; (2) the period of DNA synthesis, S_1; (3) a second growth period, $G2$, leading to mitosis. It is becoming clear, however, that mitosis is not a necessary consequence of $G2$. Thus, Gelfant[14] found that epidermal cells of mice fell into two classes, those in which $G2$ was followed by mitosis and others which appeared to be blocked in the $G2$ stage and stayed there for a long time; and Patau and Das[1] have presented evidence that cells of the tobacco plant which have synthesized DNA are ambivalent in the sense that, according to circumstances, they may either enter mitosis or go through another DNA synthesis.

It is clear that DNA synthesis unaccompanied by mitosis is synonymous with the process which has been described as 'endomitosis' or 'endoreduplication'. The term 'endomitosis' is due to Geitler[15], who observed that in the nuclei of insects of the class Heteroptera, chromosome-like structures appeared which apparently underwent an abortive mitosis. Afterwards such nuclei became polyploid. The most highly polyploid nuclei were those of the salivary gland cells, which became 64 ploid during the first instar and at least 1,024 ploid in the imago. It should be emphasized that the concept of endomitosis arose at a time when it was not yet recognized that the process of chromosome replication is essentially separate from the mitotic process. Consequently, Geitler[16] distinguishes between those cases of endopolyploidy in which the nuclei show some resemblance to nuclei in mitosis and others in which polyploidy arises without any visible symptoms apparently at a 'sub-microscopical level'. A similar distinction was made by Levan and Hauschka[17], who limited the term 'endomitosis' to nuclei which seemed to show conspicuous activity inside the nuclear membrane and gave the name "endoreduplication" to the process of chromosome replication which went on concealed during the despiralized stage. It would appear that with our present state of knowledge this distinction has lost its importance. Furthermore, the realization that mitosis does not result in chromosome replication has made the term 'endomitosis' self-contradictory, as has already been suggested[1]. The term 'endoreduplication', therefore, seems preferable to describe the process of chromosomal replication which, in the absence of mitosis, leads to polyploidy. The term 'endopolyploidy' is useful to distinguish polyploidy which has arisen in this way from polyploidy originating by nuclear fusion.

If nuclei which have undergone at least two series of replications afterwards undergo mitosis, the duplicated chromosomes characteristically occur in pairs (Fig. 2). Polyploid mitoses with paired chromosomes were first demonstrated by Levan[18] in plant cells which had become polyploid following treatment with growth hormones, and Levan and Hauschka found an abundance of such figures in ascites tumours of the mouse. Polyploid mitoses with paired chromosomes in human tissue culture cells derived from skin were first described by Fraccaro *et al.*[19]. However, the incidence of such cells in tissue culture tends to be low. As pointed out above, the incidence of all polyploid mitoses in rapidly dividing cultures is only a few per cent, and only a small percentage of polyploid mitoses have paired chromosomes. The significance of such cells is clearly that they represent the first mitotic division following endoreduplication; thus the twice-duplicated chromosomes are still more or less *in situ*, having not yet become dispersed by the movements of cell division.

The difference in the incidence of polyploidy in interphase and mitotic nuclei directs attention to the problem of nomenclature. Some authors have tried to evade the concept of polyploidy in interphase by ascribing to cells which have duplicated their DNA a certain C value[10]. Thus a diploid cell which has completed DNA synthesis would be $4C$. This nomenclature appears to be unsatisfactory for a number of reasons. The symbol C in this context does not stand for any definite term. Patau and Swift[20] wrote: "It is also generally agreed upon that these multiples, usually terms of the series C, $2C$, $4C$, show a close relation to the chromosome or rather chromatid number. C is apparently always found after the second meiotic division". There is, however, as yet no way of counting either chromosomes, chromatids or centromeres in interphase nuclei. As regards the apparent ambivalence of cells which have undergone DNA synthesis, there can be no logical objection to the statement that a cell becomes tetraploid prior to its giving rise to two diploid ones; while it is becoming clear that a substantial proportion of cells with duplicated DNA contents do not undergo mitosis but remain as interphase cells of larger size. Large interphase nuclei have been recognized as polyploid for a long time. There appears to be no reason to change this concept now that it is possible to obtain an accurate assessment of the degree of polyploidy by measuring their DNA contents.

Geitler[16] wrote that, owing to the belief in the constancy of chromosome numbers, the phenomenon of endopolyploidy was neglected. It now appears that the adherents of both apparently conflicting views were right: while



a *b*

Fig. 2. Tetraploid mitoses in human fibroblast-like cells. (*a*) Usua type; (*b*) with paired chromosomes, as is characteristic for the first mitosis following endoreduplication. (By courtesy of Dr. Joy Delhanty)

dividing cells have a strong tendency to retain the diploid chromosome number, a substantial proportion of cells become polyploid in interphase.

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PROTEIN SYNTHESIS IN RETICULOCYTES MATURING *IN VIVO*

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THE transition from mammalian reticulocyte to mature erythrocyte is marked by the loss, not only of the stainable reticular material, but also of the ability to synthesize protein. Recently, the site of protein synthesis, not only in reticulocytes but also in a wide variety of cells, has been shown to be aggregates of ribosomes called polyribosomes[1–3]. The present search for the cause of the declining rate of protein synthesis in the maturing reticulocyte was undertaken in the hope that the explanation might contribute to a general understanding of the rate determinants of mammalian protein synthesis.

Two methods have been previously used to examine reticulocyte maturation[4]. One is the comparison of cells from an animal at various times following an erythropoietic stimulus; a cell population so derived, however, is far from homogeneous. The second is the investigation of cells aged under controlled conditions *in vitro*. Using the latter method, Bertles and Beck[5] showed that maturation *in vitro* is associated with a conversion of RNA into products of low molecular weight, which are released from the cell. Marks *et al.*[6] by sucrose gradient analysis, and Rifkind *et al.*[7] by electron microscopy, showed that maturation *in vitro* is associated with a progressive disaggregation of polyribosomes to smaller clusters and single ribosomes.

The present investigation of reticulocyte maturation was prompted by the report of a method to separate red cells by age using density gradient centrifugation[8]. This method provided a means of determining whether the above conclusions about cells matured *in vitro* also applied to cells of different age removed from the same animal at the same time.

Specifically, answers were sought to two questions: (1) how does the rate of protein synthesis in the maturing reticulocyte vary with age, and (2) which component of the protein synthetic apparatus fails ? Protein synthetic rates were determined for young and old reticulocytes (*a*) as whole cells, (*b*) in cell-free systems derived from each, and (*c*) in cell-free systems in which young and old fractions were interchanged. Evidence is presented that the decline in protein synthesis in the maturing reticulocyte correlates primarily with the decline in the quantity of polyribosomes per cell.

The density of the red cell increases with age. This fact enabled Leif and Vinograd[8] to separate erythrocytes according to age on a linear density gradient of bovine serum albumin. They verified the method for rabbit erythrocytes by the serial analysis of cells obtained from a rabbit injected with $^{59}FeCl_3$.

Using position in such a gradient as a measure of age, protein synthesis in rabbit reticulocytes was determined as a function of age, first in whole cells. The experiment shown in Fig. 1 indicates that, as the cells get denser and occupy a lower position in the gradient, they are also less active in protein synthesis. In this experiment, the top sixth of the cell population was 240 times as active as the bottom sixth. What accounts for this difference between old and young reticulocytes ?

To determine this, 'young' and 'old' reticulocytes were separately pooled from the top and bottom halves, respectively, of an albumin-density gradient. The samples contained 97 and 94 per cent reticulocytes, respectively, the mature red cells from the initial sample (60 per cent reticulocytes) having formed a pellet at the bottom of the gradient tube. Both whole-cell and cell-free syntheses were examined. As whole cells, the young cells were 2·5 times more active than the old cells (Fig. 2).

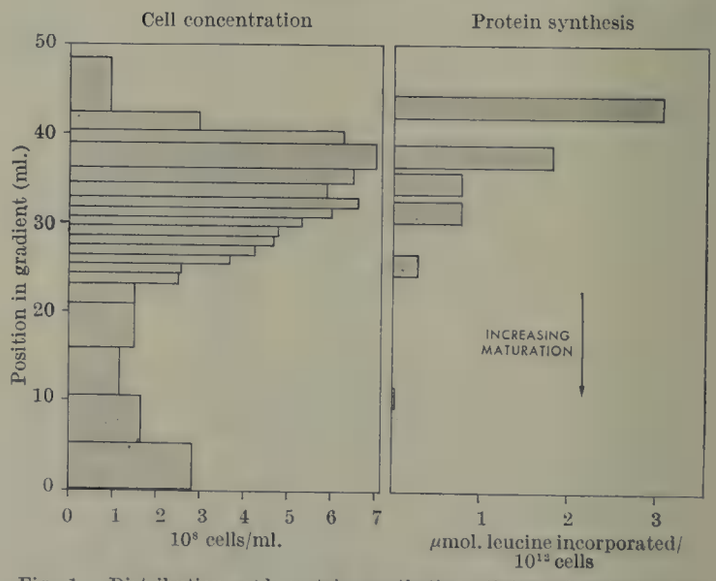

Fig. 1. Distribution and protein synthetic activity of reticulocytes separated by age on a density gradient. A New Zealand albino rabbit was injected subcutaneously with phenylhydrazine HCl (12·5 mg/kg the first day, then 10 mg/kg for four days) and bled on the sixth day. Washed, packed cells 2·5 ml., diluted to 5 ml. with saline, were layered on to a 50-ml. linear density gradient (20–35 per cent, w/w) of bovine serum albumin in saline (ref. 8) and centrifuged at 20,000 r.p.m. in a rotor type 25·2 in the Spinco Model '*L*-2' ultracentrifuge for 30 min. Six fractions were collected and the cells recovered by dilution with saline and centrifugation at 2,000*g* for 5 min. From each fraction, 0·05 ml. packed cells were incubated with L-leucine-μ-^{14}C ($3{\cdot}56 \times 10^8$ c.p.m./μmol.) 9 mμmol., other amino-acids in the ratio of Lingrel *et al.* (ref. 11) 96 μg, glucose 0·88 μmol., sodium citrate 0·18 μmol., $Fe(NH_4)_2(SO_4)_2$ 2 mμmol., NaCl 16·8 μmol., $MgCl_2$ 0·31 μmol., $NaHCO_3$ 0·26 μmol., 0·04 ml. of the same rabbit's plasma and *tris* 3·6 μmol. at *p*H 7·4 in a total volume of 0·23 ml. at 37° C for 1·5 min. The cells were then washed and lysed by the addition of 0·45 ml. of 0·01 M KCl, 0·003 M $MgCl_2$, 0·01 M *tris*-Cl, *p*H 7·4, containing 100 units of heparin per ml., and the membranes removed by centrifugation at 10,000*g* for 15 min. The acid-precipitable radioactivity of the membrane-free lysate was determined, after washing on 'Millipore' filters, using a liquid scintillation counter

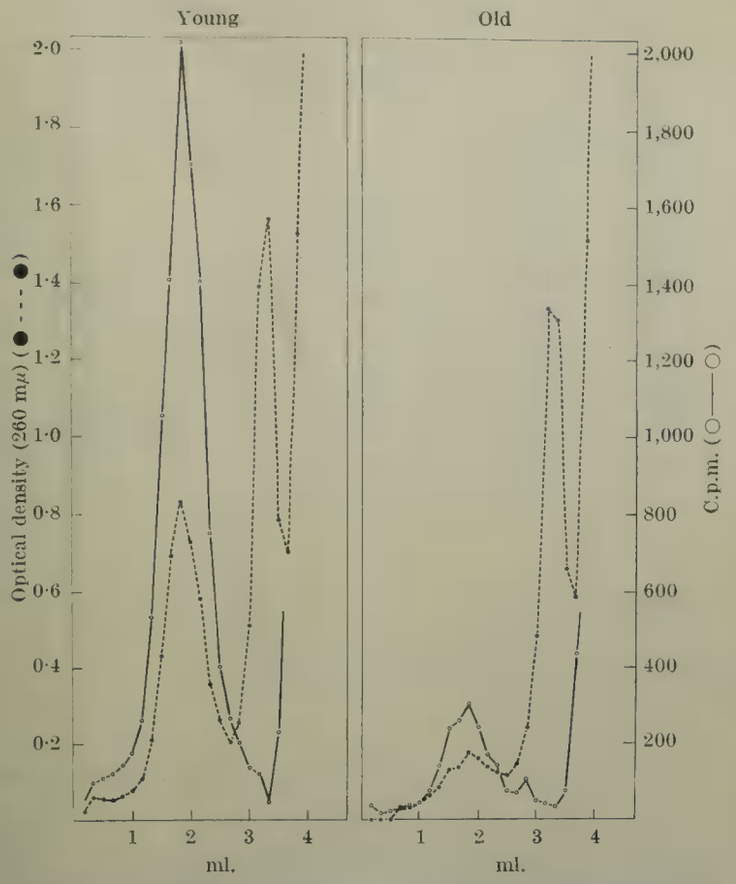

Fig. 2. The membrane-free lysates from the experiment described in Fig. 1 were used. Of the six fractions, only the analyses of fractions 2 ('old') and 6 ('young') are reproduced. Membrane-free lysate, 0·2 ml., was layered on to a 4·8-ml. gradient of sucrose, 10–30 per cent in 0·01 M KCl, 0·003 M $MgCl_2$, 0·01 M *tris*-Cl, *p*H 7·4, containing 100 units of heparin per ml. and centrifuged at 40,000 r.p.m. in a 'SW-39' rotor in a Model *L*'-2' Spinco ultracentrifuge for 1 h. Fractions, 0·25 ml., were analysed for absorbancy at 260 mμ using a Beckman '*DU*' monochromator and a Gilford 'Model 220' photometer, and for radioactivity using a liquid scintillation counter after addition of 10 ml. Bray's solution (ref. 12)

Protein synthesis by fractions from young and old reticulocytes are compared in Table 1 (incubations 1 and 2). The fractions from young cells were also several (3·8) times more active 'per cell' (see footnote) than fractions from old cells. The relative contribution of ribosomes and supernatant was determined by interchanging young and old fractions (incubations 3 and 4). The mixed incubation containing young ribosomes (No. 3) was 70 per cent as active per cell as the incubation of the wholly young fractions (No. 2), whereas the mixed incubation containing old ribosomes (No. 4) was scarcely more active than the incubation of wholly old fractions (No. 1). Hence the ribosomes were largely the rate-determining fraction.

The young cells contained more ribosomal material than the old cells. Expressed as optical density units at 260 mμ, the young cell averaged $6{\cdot}24 \times 10^{-9}$ units and the old cell $2{\cdot}49 \times 10^{-9}$ units. The greater activity of the ribosomal fraction from the young cells is due, however, not solely to their greater quantity of ribosomal material per cell, but also to a more active type of ribosomal material, as shown by a higher rate of protein synthesis per *O.D.* unit of ribosomes (Table 1).

Since protein is synthesized chiefly on polyribosomes, one might suppose that the principal difference between the ribosomal fractions of young and old cells would be in the quantity of polysomes. To test this hypothesis, young and old cells were incubated whole with radioactive amino-acids, and their lysates analysed on sucrose gradients. Young and old whole-cell fractions, separated in the experiment described in Fig. 1 and differing 10-fold in their whole-cell synthetic rate, were used. The results are shown graphically in Fig. 2 and quantitatively in Table 2. Cell for cell, the two striking differences are in the quantity of polyribosomes (old = 20 per cent of the young) and in the radioactivity in the polyribosomes (old = 14 per cent of the young). Since the quantity and radioactivity of polyribosomes are comparably reduced, their specific activity (μmol. leucine bound per optical density unit of polysome) showed only a modest decrease (old = 66 per cent young). The quantity of single ribosomes (Table 2) showed only modest reduction. The significance of the increase in average release time is not known.

Maturation of the reticulocyte is then marked by two processes, a decrease in the total quantity of ribosomes and a disproportionate decrease in the quantity of polyribosomes, as described by Rifkind[7] for maturation *in vitro* and in a peritoneal diffusion chamber. The cause of neither of these processes is known. Investigations of ribonuclease activity in lysates of young and old cells are in progress.

Maximal recovery of polysomes requires minimizing ribonuclease action during isolation. In controlled experiments, the use of heparin, a known inhibitor of ribonuclease[9], in the lysing solution and in the sucrose gradient increased polysome yields. Lysis by brief osmotic shock, described by Marks *et al.*[1] as specific for red cells, may also have been of value, since white cells have much more ribonuclease than red cells[10].

In summary, the most striking change in the maturing reticulocyte as haemoglobin synthesis decreases is a decline in the quantity of polyribosomes per cell. This conclusion has been previously reached for cells maturing *in vitro* or in a peritoneal diffusion chamber. It has now been documented for cells of different age removed from the same animal at the same time.

Since this account was prepared, a report has appeared by Glowacki and Millette (*J. Mol. Biol.*, **11**, 116; 1965)

Table 1. Protein Synthesis by Fractions from Young and Old Reticulocytes

	1	2	3	4
Source of fractions:				
Ribosomes	Old	Young	Young	Old
Supernatant	Old	Young	Old	Young
Incorporation into protein:				
μμmol. leucine	155	486	342	172
μμmol./10^9 cells*	166	639	450	185
μμmol./*O.D.*$_{250}$ ribosomes	53	82	57	59

* The calculation of μμmol./10^9 cells involved the number of cells which provided the ribosomes for that incubation.

Blood (30 ml.) from a New Zealand albino rabbit (2·8 kg), treated with phenylhydrazine HCl (10 mg/kg) on the preceding 5 days, was passed through a tightly packed column of 'Duponol'-treated nylon fibres (3 g) at room temperature to remove granulocytes (ref. 13) and centrifuged (2,000*g*, 5 min). Packed cells (2·5 ml.) were separated according to age by density gradient centrifugation (Fig. 1) and the gradient divided into two parts, an upper ('young') and lower ('old'), containing an equal volume (1 ml.) of cells after packing. Packed cells (0·8 ml.), young ($4{\cdot}57 \times 10^9$ cells) or old ($5{\cdot}6 \times 10^9$ cells), were separately lysed by the addition of 3·2 ml. 0·005 M $MgCl_2$, followed in 30 sec by 0·8 ml. 1·5 M sucrose–0·15 M KCl (ref. 1). Fractions were prepared and cell-free synthesis carried out in a manner similar to that of Allen and Schweet (ref. 14). Lysates were centrifuged at 15,000*g*, 10 min. The supernatants were recentrifuged at 78,000*g*, 1 h. Pellets were rinsed with 0·05 ml. 0·25 M sucrose, 0·0175 M $KHCO_3$, 0·002 M $MgCl_2$, homogenized in 2 ml. of the same, and ribosomes and supernatants were re-centrifuged as before. The final pellets were homogenized in 0·3 ml. 0·25 M sucrose in glass homogenizers by hand, the suspensions re-centrifuged at 15,000*g*, 5 min, and the final supernatants used as 'ribosomes'. Each incubation (0·15 ml.) contained: in μmoles, *tris*-HCl, *p*H 7·5, 56; KCl, 50; glutathione, 20; creatine phosphate, 10; ATP, 1; GTP, 0·25; $MgCl_2$, 5; ^{14}C-L-leucine $3{\cdot}55 \times 10^7$ c.p.m./μmol., 0·005; 18 other L-amino-acids, 2·1 μg total, in the ratio of Lingrel *et al.* (ref. 11); creatine kinase, 0·12 mg; supernatant, 0·04 ml., and ribosomes, 0·05 ml. After 20 min at 37°, cold water was added and the material insoluble in 5 per cent trichloroacetic acid was washed and counted on 'Millipore' filters in a liquid scintillation counter. A control incubation of young fractions to which ^{14}C-L-leucine was added after incubation incorporated a total of 80 c.p.m. Optical density of ribosome suspensions at 260 mμ was determined in 0·01 M NaOH.

Table 2. Polysomes from Young and Old Reticulocytes

	Per 10^8 cells		
	Young	Old	Old/Young
Whole cell synthetic rate μμmol. leucine incorporated/1·5 min	305	27·7	9·1%
O.D.$_{260}$mμ in polysomes	5·08	1·04	20%
O.D.$_{260}$mμ in single ribosomes	5·60	4·00	71%
Polysome-bound leucine, μμmol.	31·8	4·3	14%
Specific activity of polysomes: μμmol. leucine bound/O.D.$_{260}$ mμ	6·25	4·14	66%
Average release time, min*	0·156	0·233	149%

* Average release time is the average time radioactive amino-acid is bound to polysomes before release into supernatant: (leucine in polysomes/total acid-insoluble leucine) × 1·5 min. The data in this table were calculated from the data in Fig. 2.

describing experiments similar to some of those reported here. They also incubated intact rabbit reticulocytes, separated by age using density gradient centrifugation, with radioactive amino-acids and analysed the lysates on sucrose gradients. Despite several differences in technique, both their results and ours indicate that the decline in protein synthesis with maturation is accompanied by, cell for cell, a decrease in total ribosomal material, a decrease in the fraction present as polyribosomes without a decrease in the apparent size of the aggregate, and some decrease in the specific activity of the polyribosomes. From available data, the last mentioned could be due to either a decrease in activity of all polysomes or to a growing fraction of inactive polysomes.

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NUCLEO-CYTOPLASMIC INTERACTIONS IN THE RAT LIVER CELL

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ONE aspect of the investigation of nucleo-cytoplasmic interaction in the liver cell is the study of the intracellular movement of proteins. This requires the application of chase-labelling techniques to an *in vitro* system using a sufficiently large quantity of tissue to permit subcellular fractionation at the end of the experiment.

Whole-liver cell preparations would be ideal for these experiments, but preparations that have been described have a low yield of cells and these often have impaired metabolic activity. A metabolically active liver cell preparation such as the slice has a disadvantage in that only a limited number of slices can be prepared from each liver. For these reasons it was decided to explore the 'tissue chop' technique as a method of producing a large amount of a cellular preparation of liver.

McIlwaine and Buddle[1] designed a mechanical tissue chopper and showed that the respiration of tissue 'chops' of cerebral cortex was within 3 per cent of the respiration of ordinary hand-cut slices. They also demonstrated that such a chopper would produce tissue chops from small quantities of a whole range of tissues such as heart, liver, spleen and kidney.

In the present investigation it was necessary to be able to handle quickly at least 7 g of liver and often more. A tissue chopper was made, based on that of McIlwaine and Buddle, modified, however, to take larger quantities of tissues by increasing the size of the chopping platform and the blade. For each experiment two male rats weighing 150 g were killed by cervical fracture and the livers rapidly removed into ice-cold Krebs–Ringer-phosphate, *p*H 7·3 (Umbreit, Burris and Stauffer[2]), containing 0·1 per cent glucose. The lobes were then carefully trimmed to give slices of liver 2–5 mm. thick and chopped in two directions. The device was set to chop at intervals of 0·3 mm, thus producing minute columns of liver. The columns from 10 to 15 g liver were suspended in 30–50 ml. cold Krebs–Ringer-phosphate and allowed to sediment. The supernatant fluid containing broken cell debris was decanted and the chopped liver preparations washed three more times with cold Krebs–Ringer-phosphate before being finally suspended in Krebs–Ringer-phosphate plus glucose (30 ml. per 6–8 g chopped liver) and transferred to 100 ml. conical flasks for incubation. 10 μc. of ^{14}C-leucine was added to the flasks, which were then sealed with vaccine caps through which were passed syringe needles to allow for gas exchange. The chopped liver preparations were incubated for various times as required at 37° C under oxygen. After incubation the liver suspensions were cooled in crushed ice and washed by decantation and resuspension twice in a solution of 0·5 per cent w/v non-radiactive leucine in 0·25 M sucrose and then twice in 0·25 M sucrose. The liver preparations were finally resuspended in 0·25 M sucrose and the suspensions divided in two. One half was homogenized in a Potter–Elvehjem homogenizer for preparation of mitochondria, microsomes and cell sap as described by Schneider[3]. To the other half, calcium chloride was added to a final concentration of 5 mM and the suspension homogenized in the hand-operated homogenizer for preparation of nuclei as described previously by Rees and Rowland[4].

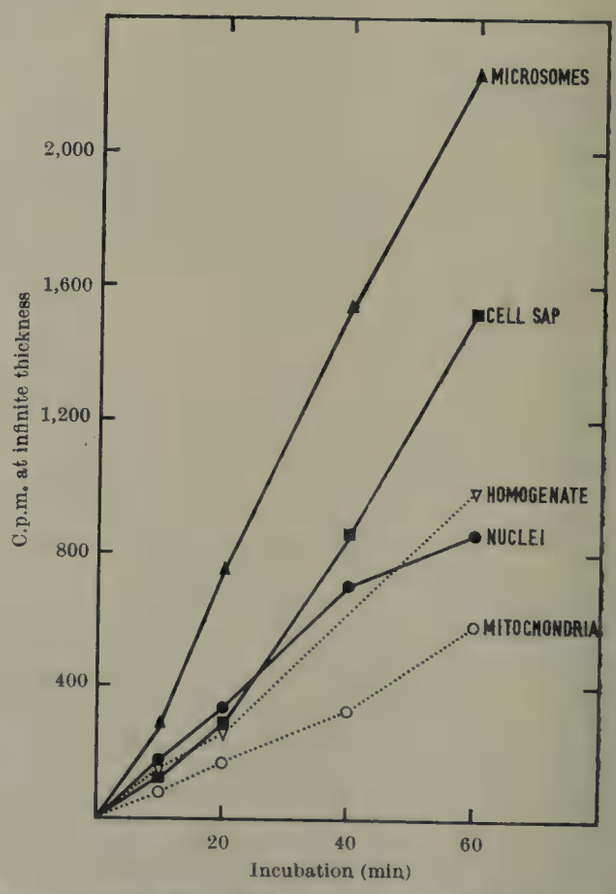

Fig. 1. Time-course of incorporation *in vitro* of 1-^{14}C DL-leucine into proteins of subcellular fractions during incubation of chopped liver preparation. Results are the means from three experiments

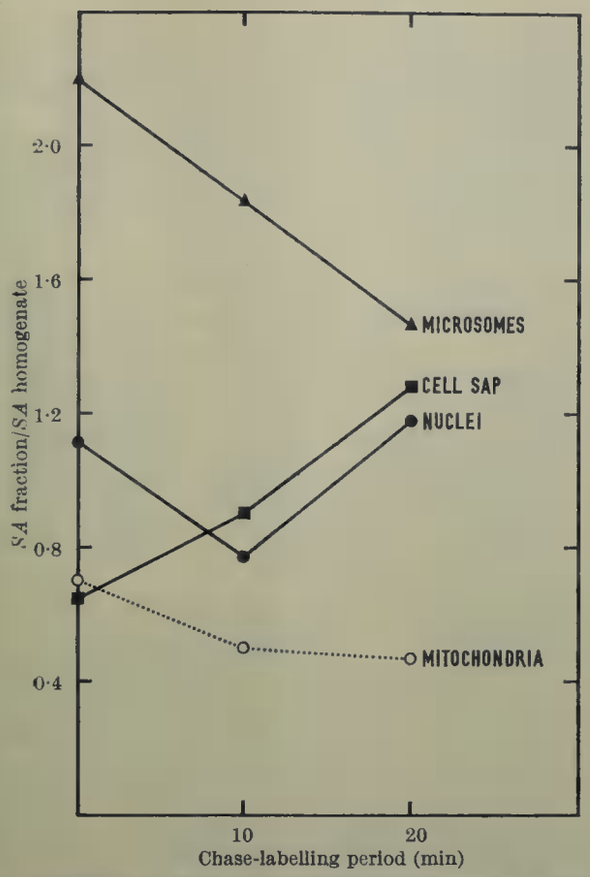

Fig. 2. Specific activities (*SA*) of sub-cellular fractions relative to specific activity of homogenate during the 'chase-labelling' period of the experiments shown in Table 1. The results are the means from four experiments

By incubating a suspension of chopped liver in a Krebs–Ringer-phosphate the time-course of ^{14}C leucine incorporation into the various subcellular fractions was examined. Fig. 1 shows the result of such an experiment. It is of particular interest to note that whereas at 40 and 60 min the cell sap proteins are more highly labelled than nuclear proteins, at earlier times this is not the case.

Several experiments of the 'chase-labelling' type were carried out in the following manner. The chopped liver preparation was washed free of broken cells in Krebs–Ringer-phosphate solution and divided into three roughly equal portions. The portions were all incubated at 37° C with ^{14}C leucine for 10 min. At this stage one portion was cooled in crushed ice while 100-fold excess of non-radioactive leucine was added to the other portions, which were allowed to continue incubating for a further 10 and 20 min, respectively, before cooling in ice also. The cooled liver suspensions were washed free of isotope and broken cells as already described and then homogenized and fractionated to yield the various subcellular components. Table 1 shows the results of a typical experiment carried out in this way. To correct for variations in the degree of overall labelling in the three portions the results have also been expressed as a ratio of the specific activity of each subcellular fraction to the specific activity of the homogenate from which they were isolated, and the means of the ratios from all the experiments are given in Fig. 2. It may be seen from these results that there is an overall fall in the radioactivity of both the microsomal and mitochondrial protein during the 20 min 'chase' period, and there is a considerable rise in the radioactivity of cell sap protein during this time. The nuclear protein shows a considerable drop in radioactivity after the first 10 min of 'chase-labelling', but during the second half of the 'chase' period the radioactivity returns to its former level.

Table 1. Incorporation of 1-^{14}C dl-Leucine by Suspensions of Chopped Liver followed by 100-fold excess Unlabelled Leucine in Order to follow Movement of Labelled Protein from Sites of Protein Synthesis within the Liver Cell

Incubation time with ^{14}C-leucine	10 min	10 min	10 min
Incubation time with ^{12}C-leucine	+0 min	+10 min	+20 min
Total time	10 min	20 min	30 min
Tissue fraction	c.p.m at infinite thickness	c.p.m. at infinite thickness	c.p.m. at infinite thickness
Homogenate	109	124	142
Nuclei	105	85	140
Mitochondria	77	57	65
Microsomes	232	195	208
Cell sap	68	112	188

Results are those of a representative experiment of a group of 4 similar experiments.

These findings are in agreement with those of Goldstein[5] and Prescott[6], whose autoradiographic investigations have shown similar movements of protein to and from the nucleus of *Amoeba proteus*. Goldstein has examined two types of nuclear proteins: proteins that migrate from the nucleus to the cytoplasm and those that do not migrate. He concluded that both types are synthesized in the cytoplasm, but his experiments do not entirely rule out the existence of a species of non-migratory proteins synthesized within the nucleus. There is at present no indication as to the function of these migratory proteins in the cell.

One possible interpretation of these results is that there may be a movement of labelled protein out of the nucleus into the cytoplasm during the early stages of incubation followed by a movement of protein in the reverse direction—from cytoplasm into the nucleus.

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BIOLOGY OF THE CYTOPLASM

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Cytoplasmic Nucleic Acids

ACCORDING to the scheme initially proposed by Gierer[1] and Noll *et al.*[2], messenger RNAs leave the cell nucleus to become individually associated with one or more ribosomes in the cytoplasm. Thereafter, the ribosomes assemble a specific protein by moving along the strand of messenger RNA, exposing by 3-base increments the codes for individual amino-acids which are linearly arranged thereon.

This concept has received abundant experimental verification from the work of Rich *et al.*[3] and others[4], and is entirely convincing for certain systems. However, many investigators have held that the foregoing mechanism constitutes only a fragment of the total protein synthesizing machinery of most of the cells of higher animals[5].

Electron micrographs of rapidly metabolizing cells offer a unique insight to this problem, and in the past we

have exploited the oocytes, follicular cells and developing organ systems of the chicken in this regard. In a recent communication[6] we reported the following observations which are pertinent to the question of how nuclear materials reach the cytoplasm and how they function therein.

(*a*) Nucleolar nucleic acid is often present in the form of small vesicles (300–400 Å diam.) possibly containing protein as well as nucleic acid. Considerable numbers of these vesicles are sometimes seen arrayed in double helical 'stacks' within the body of the nucleolus. The phenomenon may be peculiar to a given brief phase of activity or it may be that these bodies are commonly so dispersed but that fixation of nuclear materials proceeds too slowly to preserve the orientation in most cases.

(*b*) Strings of such vesicles are often seen outside the nucleolus, in the karyoplasm, one end of the string usually being in intimate association with the nuclear membrane.

(*c*) The vesicles closest to a nuclear 'pore' are observed to be markedly reduced in size.

(*d*) It appears that such smaller vesicles (microvesicles) emerge from the site of a nuclear 'pore' at the periphery of that pore—which is characterized by so-called 'annular rods'. The 'annular rods' appear to radiate into both nucleus and cytoplasm.

(*e*) It may be that the 'rods' consist of rows of very small vesicles which are continuously moving into the cytoplasm.

(*f*) In any event, the microvesicles assemble in the cytoplasm to form larger oval bodies which resemble the 'kinetosomes' of *Paramecium*[7]. At the magnifications afforded by the electron microscope we were using at the time, these bodies (1000–1500 Å smaller diam., up to 4000 Å larger diam.) appear to consist of a relatively dense membrane with regularly spaced outwardly projecting bristles (200–250 Å in length—200–250 Å apart) and a centrally located dark streak.

(*g*) Bristle-membraned bodies ('kinetosomes') were also seen to arise from the plasma membrane. We have so far not been able to distinguish these from those elaborated at the nuclear 'pores'. In the literature such have often been referred to as pinocytosis—or absorption—vesicles.

(*h*) Although we have found no way of distinguishing between the bristle-membraned bodies of differing origin once they have entered the cytoplasm proper, some such bodies in the cytoplasm were observed to be in various stages of condensation into—or synthesis of—denser granules. The most advanced appeared to be in transition from vesicles to ribosome clusters.

(*i*) Many of the bristle-membraned bodies were seen to be participating in the formation of yolk granules in the oocyte, and vesicles of various sizes were conspicuous among other developing cell organelles, with and without transformation into ribosomes or polyribosomes.

Since we reported the foregoing findings, an electron microscope of higher resolution (Siemens 1*A*) has been installed in our laboratory and we have examined numerous additional samples of these avian tissues with this more powerful instrument. Thinner sections (400 Å) have been made with a diamond knife mounted on a Porter–Blum *MT*2 microtome. As was done previously, eggs and embryos were fixed by exposure to 1–2 per cent osmic acid (*p*H 7·8) for 2 h, were taken up through 50, 75, 95 and 100 per cent ethyl alcohol steps to propylene oxide and were then embedded in 'Epon 812'. Copper grids without support were used and staining with lead was carried out according to method *B* of Karnofsky[8].

One of the more gratifying results of this new investigation was the confirmation of something we had suspected earlier, namely, that the bristle-membraned bodies (or 'kinetosome-like' bodies) are largely constituted of small vesicular units of between 150 and 250 Å outside

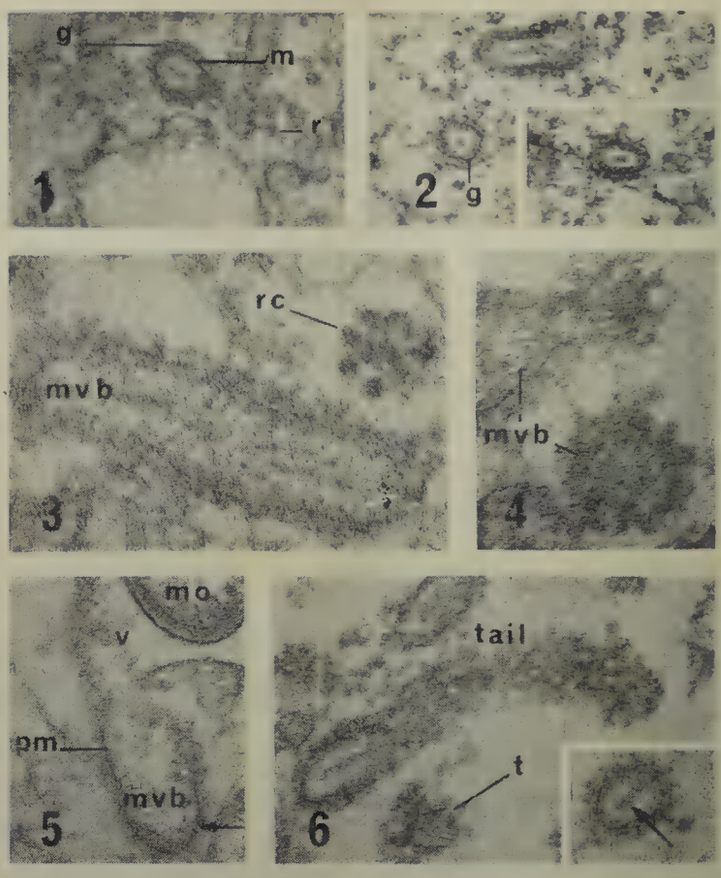

Fig. 1. Multivesicular body within cytoplasm of 4 mm hen's egg showing outer sheet of vesicles with small internal granules (*g*), membrane (*m*) between outer sheet and inner closely packed sheet of vesicles, 'honeycomb'-like interior with separations composed of small granules or microvesicles and central streak consisting of either granules or microvesicles. A cluster of ribosomes appears to be associated with this body and may be derived from it. (×56,000)

Fig. 2. Multivesicular bodies from cytoplasm of 1·8 mm hen's egg showing relative sizes of vesicles in shells separated by dense membrane. Note that vesicles of internal sheet also contain very small granules (*g*). (×50,000)

Fig. 3. Elongated multivesicular body (*mvb*) and ribosome cluster (*rc*) from 6-mm egg. Note that ribosomes are enmeshed in mass of less dense material and are about the same size as larger surface vesicles of multivesicular body (*mvb*). (×120,000)

Fig. 4. Multivesicular body (*mvb*) with 'tail' which may be condensing into ribosomes (top) and multivesicular body (*mvb*) sectioned through surface sheet of vesicles (bottom). Note dense granules marking centres of vesicles. 6 mm egg (×120,000)

Fig. 5. Multivesicular body apparently budding off from vesicle (*v*) carrying 'mitochondrion-like' organelle (*mo*) into 6 mm hen's egg. The plasma membrane (*pm*) appears to be homologous to the membrane of the multivesicular body (arrow). (×80,000)

Fig. 6. Multivesicular body in 6-mm hen's egg with elaborated 'tail'. This suggests that the body may either be (*a*) duplicating itself, (*b*) in an early stage of forming ribosomes or (*c*) both. A section through the surface layer of a multivesicular body is shown (*t*) which clearly reveals the vesicular orientations of this sheet and the central location of the granules. The arrow (insert) points to microvesicles which sometimes appear to comprise the central streaks of these bodies. (×80,000)

diameter (Figs. 1 and 2). The 'bristles' then, seen at lower resolution, have proved to be the sites at which the sides of adjacent microvesicles are pressed together. It is, however, not clear whether material in addition to that contained in the two membranes may be present at these junctions.

The so-called limiting membrane of the multivesicular body lies between two layers of tightly packed vesicles. In Figs. 1 and 2 it can be seen that the internal layer is made up of somewhat smaller vesicles (about 150 Å) as opposed to those of the external layer (about 200 Å). Figs. 4 and 6 show a cut through the surface layer of one of these bodies and confirm the notion of the regular size and disposition of the microvesicles. Note the presence of small 'granules' in the microvesicles. In Figs. 1 and 2 it can be seen that the 'granules' are present inside the microvesicles of both surface layers. These specks are so small (about 40 Å) that they do not fulfil the

spatial requirements for a nucleic acid molecule of any respectable size. At the most they can represent only a few nucleotides and, indeed, they might consist of some altogether different substance. The so-called 'limiting membrane' of the multivesicular body stands out quite distinctly as can be seen in Figs. 1–4. This does not seem to be due to reinforcement due to the pressing together of the sides of adjacent microvesicles since, as is seen in Fig. 5 (arrow), the 'limiting membrane' appears to be the actual plasma membrane in those cases where the multivesicular bodies arise from the surface of the cell. The 'membrane' affords a stronger contrast and appears thicker than the vertical regions between the microvesicles and seems somewhat granular. It may be a major site of nucleic acid concentration in these bodies. As we have pointed out before[6], these bodies display a considerable contrast with the background following destaining with hydrogen peroxide—indicating the presence of a dense material such as nucleic acid. They also stand out in sharp contrast following application of periodic acid and silver methionine as do other nucleic acid-containing structures.

In Figs. 1–3 the impression is obtained that microvesicles organized into somewhat larger wedge-shaped structures 'honeycomb' the interiors of the multivesicular bodies. The dense central streaks in these bodies sometimes appear to be a collection of very small microvesicles (Fig. 6, arrow). The relative thickness of their walls could account for the dense streak but they may also be condensed into granules.

Conversion of some multivesicular bodies to individual dense objects morphologically indistinguishable from ribosomes and to clusters of ribosomes is strongly suggested by Figs. 7*a*, *b*, *c* and *d*, and 8. In Fig. 7*a* several ribosomes are seen extending from a partially condensed, but none the less clearly recognizable, multivesicular body. The 'stalks' appear to consist of very small microvesicles. Fig. 7*b* shows another such disposition of ribosomes but the multivesicular body is itself beginning to display solid dense nodes. A cut through the surface layer of microvesicles of another multivesicular body (Fig. 7*c*) shows that many of the microvesicles seem to be filled in with a dense material. Three nearly completed ribosome clusters are seen in Fig. 7*d*. Note that the cluster at the upper right still retains the orientation imposed by the original multivesicular body. In Fig. 8 it appears that entire ribosome clusters may arise from multivesicular bodies without disintegration of these bodies. It is possible that multivesicular bodies even duplicate themselves (body with the long 'tail' in Fig. 6) and these bodies could in turn give rise to ribosomes and ribosome clusters.

Still another piece of evidence for the conversion of multivesicular bodies—or at least cytoplasmic vesicles—into ribosomes is the observation that cytoplasmic

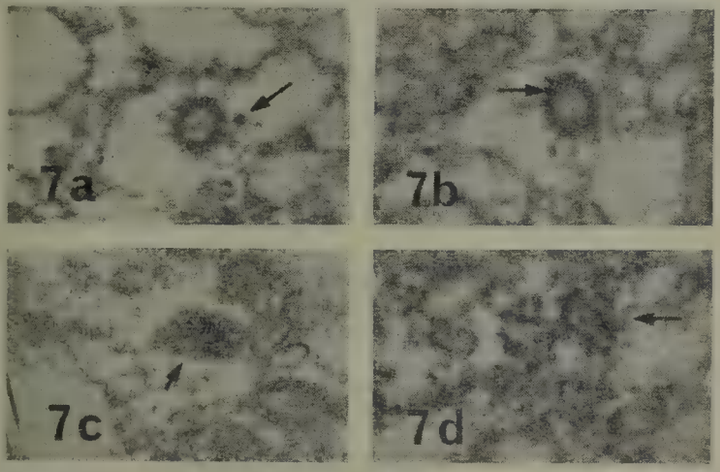

Fig. 7 *a*, *b*, *c*, *d*. This sequence suggests one mode of evolution of ribosomes and ribosome clusters from multivesicular bodies. (×46,000)

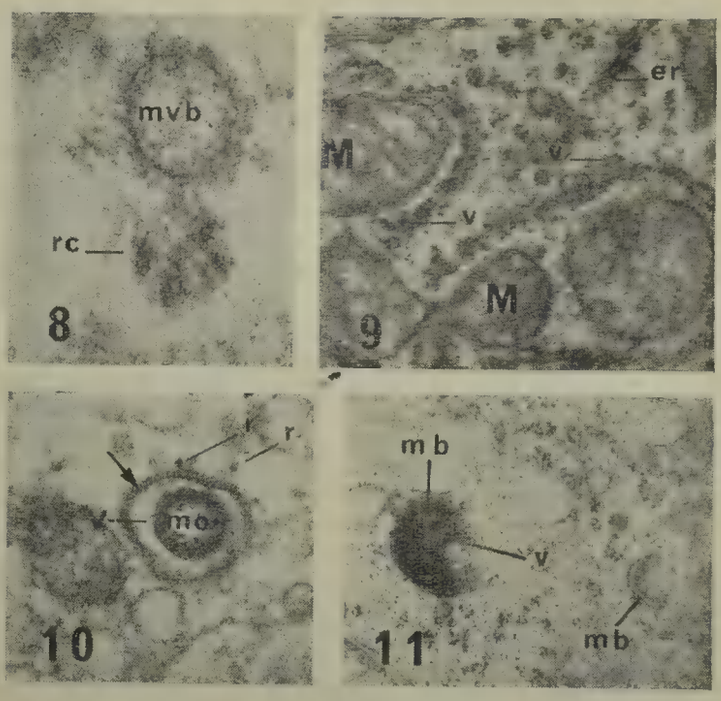

Fig. 8. The connexion between this ribosome cluster (*rc*) and the multivesicular body (*mvb*) suggests that one was derived from the other. It seems likely that a 'tail' of more or less organized vesicles was elaborated as in Fig. 6 and that this tail then condensed to give a ribosome cluster. Note vesicular nature of ribosomes and vesicular mass in which they are enmeshed. (×138,000)

Fig. 9. Endoplasmic reticulum (*er*) forming around mitochondria (*M*) in liver of 6-day-old chicken embryo. Note apparent joining of ribosome-studded vesicles (*V*). (×25,000)

Fig. 10. Ribosomes (*r*) apparently forming from vesiculated surface (arrow) of invaginated pocket of plasma membrane (*V*) carrying 'mitochondrion-like' organelle into cytoplasm of 6 mm hen's egg. (×42,000)

Fig. 11. A general darkening in a mitochondrial bud (*mb*) and the accumulation of vesicles (*v*) are associated with the maturation and increase in size of a mitochondrion somatic tissue of a 1-day-old chicken embryo. (×26,000)

vesicles in embryonic cells sometimes appear to fuse or elaborate into endoplasmic reticulum. This characteristically occurs at a more-or-less regular distance from a newly developed mitochondrion. Not only is the resulting endoplasmic reticulum studded with ribosomes but dense granules sometimes appear in the migrating vesicles prior to complete incorporation into the reticular system (Fig. 9).

For a time we thought that multivesicular bodies arising from the plasma membrane, although thus far morphologically indistinguishable from such bodies elaborated from the nuclear pores, might not display the apparent capacity for producing ribosomes[15]. However, further investigations in the oocyte have revealed that the plasma membrane often is converted in localized regions into a layer (or layers) of microvesicles (Figs. 5 and 10). It is from these regions that the multivesicular bodies of plasma membrane origin appear to be elaborated inward into the cytoplasm. As can be seen in Fig. 10, individual ribosomes are sometimes connected to the vesicular surface of the plasma membrane by thin, probably vesicular, strands. Because of the similarity of this orientation to that observed in those multivesicular bodies undergoing the first stages of differentiation into ribosome clusters (Fig. 7*a*), we entertain the possibilities: (*a*) that they may be elaborated from materials within the plasma membrane or (*b*) synthesized under the direction of—or in association with—this vesicular membrane.

We have no unequivocal proof that the multivesicular bodies specifically arising from the nuclear pores also give rise to ribosomes and ribosome clusters. However, incompletely developed ribosome clusters have been seen associated with nuclear forces. Moreover, in view of our theory that nucleolar nucleic acid is exported from the nucleus to the cytoplasm in this physical form and from the evidence presented by Edström[9] that nucleolar RNA has

a similar base ratio to ribosomal RNA, this seems likely. Also, the extreme similarity in morphology between multivesicular bodies arising from nucleus and plasma membrane suggests the existence of this degree of similarity of function.

It is, to say the least, challenging to attempt correlation of the foregoing observations with recently proposed schemes for nucleic acid participation in protein synthesis by Gierer[1], Noll *et al.*[2] and Rich *et al.*[3], in which the individual ribosomes are considered as single manufacturing units transcribing information for amino-acid sequence by movement from one end of an *m*RNA strand to the other. The nature of the evolution of the ribosome clusters in avian material suggests that the connexions from one ribosome to the other is derived from material originally present in the multivesicular precursor or at least from materials elaborated *in situ*. Although this connecting material may be *m*RNA already incorporated into the multivesicular body, the presence of as many as 20 ribosomes in such a cluster, the apparent linking of many ribosomes in more than two directions (indeed, by a mass of material in some cases, Figs. 3 and 8) and the presence of a centrally located ribosome in the cluster as well as encapsulating membrane material in some cases (Figs. 3 and 8), would seem to preclude the possibility of this unit acting in the manner proposed by the foregoing authors, namely, the simple movement of ribosomes in a single direction along a strand of *m*RNA.

Perhaps the ribosome clusters as such are not actively participating in protein synthesis. We have seen many examples in which individual ribosomes are attached by the thinnest of threads to the main mass of a multivesicular body. These may detach from the main body and thus become available to associate with a strand of messenger RNA forming a 'true' polyribosome. Following an essentially similar principle the entire ribosome clusters might disintegrate, again releasing individual ribosomes.

On the other hand, the ribosome—*m*RNA read-off—concept is difficult to apply to protein synthesis as it takes place in the plasma membrane, in growing mitochondria (Fig. 11) and in endoplasmic reticulum. Few, if any, such classical orientations of ribosomes and *m*RNA can be seen in these structures although it might be assumed that the ribosomes on the outside surface of the endoplasmic reticulum are reading off a code from *m*RNA in, or applied to, the membrane. Nisman and Pelmont[5] have pointed out that careful analysis of several systems forces the conclusion that a complete *de novo* synthesis of protein does not occur in material lacking membrane or lipoprotein and that ribosomes are usually associated with such systems. Henshaw *et al.*[10] have demonstrated that the major active unit for amino-acid incorporation in microsomal materials is, however, much larger than a ribosome cluster. In the livers of roosters that have received pharmacodynamic doses of oestrogens and are consequently synthesizing large amounts of phosphoproteins, we see relatively few ribosome clusters but many more ribosomes associated with a hypertrophied endoplasmic reticulum.

The apparent participation of multivesicular bodies and smaller vesicular structures which may be derivatives of multivesicular bodies in formation of yolk granules, Golgi complex, mitochondria and plasma membrane[6] as well as cilia[7], implies that guidance of protein incorporation into definite structure (and, probably, synthesis of protein) can be accomplished by some property of certain vesicular bodies and does not directly require the presence of ribosomes. Messenger RNA may already be present in multivesicular bodies or may associate with them in the cytoplasm and consequently may also be present in or about the structures into which the vesicles are incorporated. In these circumstances, the *m*RNA may be transcribed by some process other than the movement of granular ribosomes. Beljanski *et al.*[11] have demonstrated the presence of an RNA in *Alcaligenes faecalis*, which accepts amino-acids directly in a ratio of one amino-acid per 6 nucleotides. Existence of DNA as well as RNA has been demonstrated in plasma membrane[12] mitochondria[13] and *E. coli* spheroplast membranes[5]. Labelling with ^{14}C actinomycin *D* has revealed the presence in the cytoplasms of developing frog's eggs of considerable amounts of DNA, largely contributed from the surrounding follicular cells. These findings, taken together with the foregoing observations, lead to the conclusion that although a variety of protein-synthesizing mechanisms may obtain in the same cell, multivesicular bodies, the ultimate origins of which would be expected to be nuclear, either that existing in the present cell, one contained in a contributing cell, or one which existed in a relatively close ancestor, may be of paramount importance. By means of such bodies, elements necessary for protein synthesis, disbursement or both, may possibly be sent, stored, duplicated, transcribed into structure and, perhaps, often re-transcribed into such mobile units, according to the demands of the immediate environment.

Balbiani Ring Particles in the Cytoplasm ?

In the cytoplasms of oocytes which have attained a size of about 1·5–2·0 mm but not in eggs larger than 4 mm diameter, we have consistently observed the presence of largish (about 300 Å) particles. These occur singly for

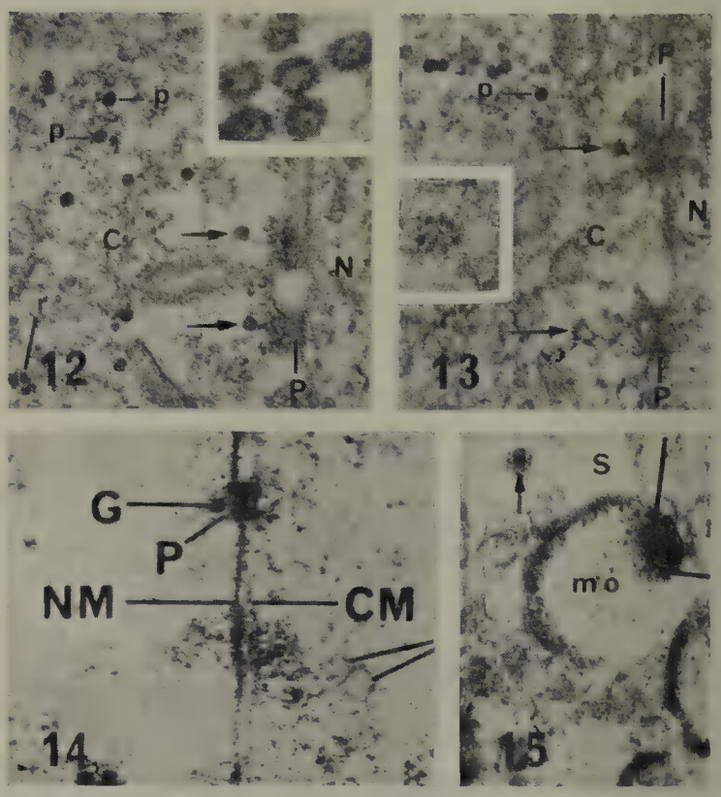

Fig. 12. The 300-Å particles (*p*) seen in the cytoplasm (*C*) of this 2-mm egg appear to have emerged from the nuclear pores (*P*) (see arrows for 2 examples), but the particles usually observed in the centres of these pores are always smaller (see tangential cuts through such pores in insert). The size, appearance and apparent origin of the larger particles suggest that they may be Balbiani ring granules containing messenger RNA. *r* = ribosomes. *N* = nucleus. (× 50,000)

Fig. 13. These two examples of 300-Å particles apparently having just passed through the nuclear pores (arrows) indicate either that the particles re-form from several smaller particles after entering the cytoplasm or that they sometimes unravel in the cytoplasm. It will be noted that even in the condensed form they appear to consist of several dense subunits. The insert shows the relative size of ribosomes, here arranged in a cluster. (× 50,000)

Fig. 14. A presumed Balbiani ring granule (*G*) is depicted here, apparently in the process of entering an annular, or nuclear, pore. *NM* = nuclear membrane. *CM* = cytoplasmic membrane. (× 55,000)

Fig. 15. Many such 300-Å particles (enclosed in a matrix or membrane sheath, arrow) are seen in the intercellular spaces (*S*) between follicular cells at the time of rapid synthesis of 'mitochondrion-like' organelles (*mo*) by these cells. (× 50,000)

the most part and appear to be slightly more dense in their centres and along their peripheries (Figs. 12 and 13). In some cases they appear to be condensed into several connected sub-granules. Although detectable in the karyoplasm, they are present there in much less concentration than in the cytoplasm, a situation which may be due to relatively poor fixation of the interior of the nucleus. In many cases (Figs. 12 and 13) they are so aligned with the nuclear pores that they give the impression of having emerged from these. In Fig. 14 similarly sized particles are observed on the nuclear side of the nuclear pores. Although it is possible (perhaps even probable) that they are essentially identical with the granules observed to be present in most of the apertures of the pores (Fig. 12, insert), few instances have been seen in which the granules in the pores are as large as the average such particles in the cytoplasm. Perhaps, as is suggested in Fig. 13 (arrows) an at least partial unravelling of the granule occurs during passage through an annular 'pore'. In morphological appearance, mode of dispersion and size, these granules closely resemble the particles that originate at the sites of the Balbiani puffs, or rings, on chromosomes[14]. Because of the site of their origin and the base ratios of the RNA associated with the Balbiani puffs[9] the so-called Balbiani ring particles are thought to consist largely of messenger RNA. Thus, the observed particles in the cytoplasm of oocytes of this stage may represent messenger RNA.

If these particles are indeed Balbiani ring particles this may be the first observed instance of such bodies appearing in compact form in cytoplasm. Beermann and Clever have shown them in the process of passing through the nuclear 'pores' of giant cells in Diptera, but in this case they appear to assume a more dispersed or thread-like form immediately on entry into the cytoplasm[14].

As mentioned earlier, we have seen these granules in the cytoplasm of the oocyte at only one stage of maturation (1·5–4·0 mm egg size). It is possible that they are *en route* to cytoplasmic or plasma membrane sites where they can be incorporated for future utilization. On the other hand, they may be participating in some function that is peculiar to, or being originated at, this stage of development. A most conspicuous such process is the formation of 'mitochondrion-like' organelles[16]. Although these are synthesized at the surfaces of the follicular cells, it is suspected that the 'messages' for such syntheses originate in the oocyte. Their ultimate destinations are, indeed, the yolk granules of the developing egg. We have long been puzzled by the occasional presence of dense granules (about 300 Å) enclosed in what appear to be envelopes of plasma membrane, in the intercellular spaces (Fig. 15). Is it possible that these granules are Balbiani ring particles carrying information to the surfaces of the follicular cells from the oocyte ?

If this be so, a logical explanation is forthcoming for the occurrence of Balbiani ring granules in a condensed form (resistant to RNases) in the cytoplasm of the small oocyte. The condensed form of the messenger might provide an efficient 'capsule' for the transport of information through a region where not only is it not required but where it might also be detrimental. Inherent in this concept is the principle that condensed *m*RNA is recognized by, or recognizes, the site where it is to unfold and carry out its function.

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15 Roth, T. F., and Porter, K. R., *Fifth Intern. Cong. Electron Microscopy* (1962), suggest that the movement of multivesicular bodies (their "specialized sites on the cell surface for protein uptake") from the cell surface to the developing yolk granules constitutes circumstantial evidence that this is the form in which yolk proteins are carried from the circulation to the yolk granule. In this connexion we have noted that the surface sheet of vesicles of the multivesicular body are usually stripped off before this body joins the yolk granule. However, the vesicular nature of the internal sheet and the centrally located granules (Figs. 1, 2) suggest that at least this layer is also not made up of yolk proteins. It is possible that a major function of the stripped multivesicular bodies is to provide nucleic acids to the yolk granules (these are known to be present therein) but this question (and the question of yolk protein transport by these bodies) is yet to be resolved.

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BREAKDOWN RATE OF SERUM 7S γ-GLOBULINS IN MICE

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THE turnover rate of γ-globulin has been determined in a number of animal species by means of radioisotopically labelled protein. The rate of γ-globulin catabolism can be influenced by several factors. An investigation by Fahey *et al.*[1] on mice indicates that these factors include the metabolic rate of host, specific features of the protein molecule, the serum γ-globulin level and, indirectly, the rate of synthesis of γ-globulin. The production of antibodies might also be expected to confer a different catabolic rate on the γ-globulin fraction containing the antibody.

Preliminary work has shown that the antibody response in mice to hyperimmunization with homologous transplantable tumours is confined to the 7*S* γ-globulin fraction[2]. Ultracentrifuge investigations have revealed appreciable increases in molecular heterogeneity of 7*S* γ-globulin samples isolated from mice immunized with homologous transplantable tumours, as compared with 7*S* γ-globulin samples from non-immunized mice of the same strain[3].

In view of the wide range of electrophoretic mobilities of 7*S* γ-globulin of mice and other species, it is difficult to believe that all the 7*S* γ-globulin molecules arise from the same site or are broken down in an identical fashion. It is therefore of interest to know whether there are differences in the breakdown rates of normal and hyperimmune serum 7*S* γ-globulins and whether fractions of these 7*S* γ-globulins, with different mean electrophoretic mobility on starch gel, also show differences.

Four series of experiments were carried out and details of strain and sex of the mice are tabulated in Table 1. Batches of about 50 normal mice of each strain were bled

Table 1. Mean Biological Half-life Values of Mouse Serum 7S γ-Globulins as determined by Different Methods

Exp. No.	Strain	Sex	No. of mice	Half-life (days) Body count method	Serum method	Starch-gel method
Normal mice						
1	Albino	M	6	—	4·6 ± 0·37	4·4 ± 0·29
2	Albino	F	6	4·5 ± 0·16	4·3 ± 0·15	4·2 ± 0·17
3	129	3 M, 3 F	6	4·4 ± 0·29	—	3·9 ± 0·25
Immune mice						
4	129	4 M, 4 F	8	5·0 ± 0·21	5·6 ± 0·35	5·1 ± 0·23

from the tail vein to provide normal sera. Immune serum was obtained from a batch of 50 mice 129 strain immunized with a transplantable mouse sarcoma *BP*8. The immunizing tumour *BP*8 was kindly supplied by Dr. J. R. Batchelor, of Guy's Hospital Medical School, London, and this was maintained in this laboratory in strain *C57BL* mice. The mice received three subcutaneous injections over a period of about 3 months of the freshly homogenized tumour, collected from the *C57BL* mice. The sera of these mice showed considerable agglutinating activity against *C57BL* red cells at the end of this period.

7*S* γ-globulin was isolated from the normal and the immune sera by chromatography on Whatman DEAE-cellulose[3]. These preparations appeared to be free from other serum proteins as shown by starch gel-electrophoresis, immunoelectrophoresis (using rabbit anti-mouse serum antiserum) and by sedimentation velocity analysis[3]. Autoradiography after immunoelectrophoresis of preparations labelled with iodine-131 revealed some contamination with β-globulin. However, only labelled γ-globulin could be detected by this technique in serum taken from the mice 5 days after injection of the labelled γ-globulin.

The γ-globulin preparations (10 mg/ml.) were labelled with iodine-131 according to the method of McFarlane[4], without preoxidation, and diluted with isologous mouse serum to produce solutions with activities of 100–200 μc./ml. 0·2–0·5 ml. of these solutions were injected intraperitoneally into normal mice of the isologous strain. Before and during the experiment, the mice were given drinking-water containing sodium iodide. The total amount of γ-globulin, labelled and unlabelled, injected into each mouse was of the order 2–10 mg. This additional γ-globulin would not be expected to have any significant effect on the rate of γ-globulin catabolism.

Radiation counts were made using an annular sample arrangement with a 15-mm cylindrical sodium iodide crystal and a Panax 'Dekatron' scaler type *D* 657. *C*. The total decay of the labelled protein was followed in three ways. These were: (*a*) total-body counts; (*b*) serum counts; (*c*) counts of the γ-globulin fraction of the serum after separation by starch-gel electrophoresis. The latter is referred to as the starch-gel method in this article.

For method (*a*) each mouse was lightly anaesthetized with ether and introduced into a Paxolin tube symmetrically situated between four integrated Geiger–Müller probes. For methods (*b*) and (*c*) 0·2 ml. of blood was obtained from each mouse by bleeding from the tail vein[5] at suitable intervals of time. Serum from each mouse was separated individually and 0·02 ml. volumes were diluted with 10 ml. normal saline for counting. Further 0·02 ml. serum samples were electrophoresed on starch gel. After electrophoresis the starch gel was sliced horizontally and both halves stained with amido black. Two fractions of the γ-globulin zone were cut out from each half of the gel. One fraction, designated *S*γ, was cut out between the loading origin on the gel and the stained $S\alpha_2$ globulin band. The other fraction, designated *F*γ, was the remainder of the γ-globulin up to, but excluding, the β-globulin bands. Measurements showed that the radioactivity was almost exclusively confined to the stained γ-globulin zone. Not more than 1 per cent of the overall activity was associated with the β-globulin bands and some of this was probably due to γ-globulin contamination. The gel fractions were each dissolved in 10 ml. N sodium hydroxide and counted.

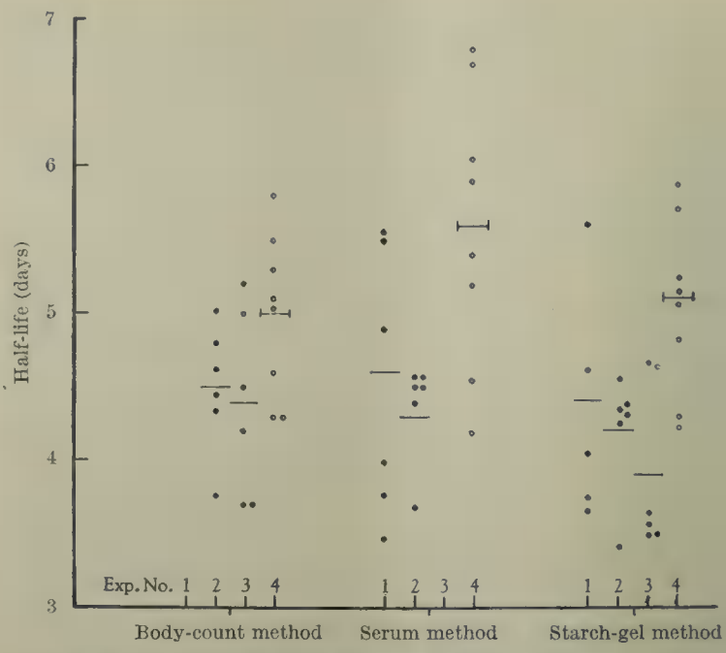

Fig. 1. Biological half-life values of the serum 7*S* γ-globulins of individual mice as determined by different methods. ●, Normal mice; ○, immune mice; —, mean values of half-life for normal mice; ⊢, mean values of half-life for immune mice

The radioactivity counts obtained by each method were corrected for normal isotopic decay. Activity measurements were made 4 or 5 days after injection of the dose and were continued for up to 22 days. Biological half-life values were computed graphically for individual mice from plots of $\log_{10}$ radioactivity against time. These half-life values were averaged and the standard error evaluated for each experiment and for each method (Table 1). The individual values are shown in Fig. 1.

These experiments suggested that there was no difference in the rate of γ-globulin turnover between the two sexes. In order to verify this, 15 male and 15 female mice of strain 129 were injected with labelled normal γ-globulin and the rate of breakdown followed by the body-count method. Average half-life values of 4·6 ± 0·11 and 4·9 ± 0·17 days were obtained respectively for male and female mice. The difference between these values is not statistically significant.

The values calculated from body and serum counts were in close agreement with the starch-gel values. The starch-gel method provided three half-life values for γ-globulin, corresponding to the turnover rates of the total 7*S* γ-globulin (*T*γ), and *S*γ, and the *F*γ fractions. Table 2 shows the average half-life values obtained by the starch-gel method. The difference in rate of turnover between the *S*γ and *F*γ globulin fractions of normal γ-globulin was statistically highly significant.

In normal iodine-131-labelled γ-globulin preparations the initial ratio of the radioactivity in the two fractions, expressed as *S*γ/*F*γ, was about 1·5–2. However, with labelled immune preparations, produced from two different strains of mice immunized with different transplantable tumours, ratios of about 6–8 were observed. Similar relative increases in concentration of a slow electrophoretic fraction of the γ-globulin have been observed semi-quantitatively in the immune response of the mouse to other antigens, and agglutinating antibody was largely confined to this slow fraction[6]. The extremely low level of radioactivity associated with the *F*γ fraction of the immune γ-globulin preparation made it difficult experimentally to obtain a value of the half-life of this fraction with any degree of accuracy.

Table 2. Mean Biological Half-life Values of Normal Mouse Serum 7*S* γ-Globulin Fractions

		Half-life (days)
Total 7*S* γ-globulin	(*T*γ)	4·2 ± 0·12
Slow fraction	(*S*γ)	3·6 ± 0·14
Fast fraction	(*F*γ)	4·6 ± 0·16

The immune γ-globulin preparation had a longer half-life in normal mice than the corresponding normal γ-globulin preparation. This difference was highly significant statistically. This effect might have been produced by the presence of long-lived impurities in the immune preparations which were absent in the normal preparation. A similar consideration could, of course, apply to the *S*γ and *F*γ fractions. There was no evidence, however, to indicate that the immune γ-globulin was significantly less pure than the normal γ-globulin. Since β-globulins had been detected as minor impurities in both preparations by autoradiography, it was possible that the differences in catabolism rate might be correlated with small differences in the level of this impurity. Mouse β-globulins were isolated from serum using DEAE-'Sephadex' chromatography[7]. Preliminary experiments showed that the rate of turnover of β-globulin in the normal mouse was very similar to that of 7*S* γ-globulin, indicating that β-globulins were not responsible for any of the observed differences.

It was found necessary to use freshly isolated γ-globulins from fresh sera or sera stored at − 20° C for only a short time. γ-Globulin isolated from serum which had been stored at − 20° C for 18 months behaved normally on starch-gel electrophoresis, immunoelectrophoresis and sedimentation velocity analysis. However, when labelled and re-introduced into mice, it was found to have a half-life of only two days. The fact that the decay of this γ-globulin was constant for some 20 days shows that this was not denatured protein in the usual sense. It seems probable that this γ-globulin had undergone some degree of limited aggregation. Such aggregation phenomena in stored γ-globulin preparations have been reported by other workers[8,9]. Limited aggregation of the molecules, *in vivo* or *in vitro*, may be responsible for the observed differences between the *S*γ and *F*γ fractions.

In summary, these studies indicate a mean biological half-life of about 4·2 days for normal mouse γ-globulin. The turnover rate did not appear to be influenced by either the sex or the strain of the mouse. The use of starch-gel electrophoresis for separating the labelled γ-globulin has made it possible to examine the rates of turnover of two fractions of the γ-globulin. These fractions, corresponding to molecules migrating with different mean mobilities when electrophoresed, had mean half-lives of 3·6 days (*S*γ) and 4·6 days (*F*γ) respectively. The fractions are probably identical with those identified by Fahey on the basis of immunological differences and on their electrophoretic mobility on starch-gel.

Immunization of 129-strain mice with transplantable tumour type *BP*8 produced hyperimmune γ-globulin which had a longer half-life than the corresponding normal γ-globulin preparation when re-introduced into normal 129-strain mice. The reasons for this are unknown, but it appears that this effect is not produced by long-lived impurities in the immune γ-globulin preparation. The considerable increase in the radioactivity ratio, *S*γ/*F*γ, of immune γ-globulins as compared with normal γ-globulins may indicate a specific response to immunization in some fraction of the 7*S* γ-globulin and not in the 7*S* γ-globulin as a whole.

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LABELLING OF MOUSE THYMOCYTES *IN VIVO* WITH TRITIATED THYMIDINE FOR CELL TRANSFER EXPERIMENTS

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IN experiments designed for the tracing of tritiated thymidine-labelled donor thymocytes after injection into a recipient animal it is of importance that the donor cell suspension should have a high percentage of labelled cells. In autoradiographic detection of transfused cells, a reasonably high grain count per cell is also desirable.

After a single injection of tritiated thymidine 15–20 per cent[1] and 6·1 per cent[2] of mouse thymocytes are reported to have been labelled, and 7–17 per cent[3] of guinea-pig thymocytes.

In vitro culturing of thymocytes in a medium containing tritiated thymidine has also been applied. With this method 9 per cent of rat thymocytes[1], 6·8 per cent[4] and 7–12 per cent[5] of guinea-pig thymocytes, and 6·7 per cent of mouse thymocytes[2] were found to be labelled.

Repeated injections of tritiated thymidine *in vivo* have given a higher yield of labelled cells in the thymus. Everett *et al.*[6], in rats, obtained a proportion of labelled thymocytes of 50 per cent after injections every 4 h for 36 h, and Murray *et al.*[7] report a proportion of 50 per cent after three intraperitoneal injections. In young mice Mims[8] found a maximum count of 54 per cent after six injections every fifth hour.

In the present study, inbred *CBA* male mice were used. Tritiated thymidine (Schwarz) with a specific activity of 3 c./mmole was diluted in nine volumes of sterile saline to a concentration of 0·1 mc./ml., and 1 or 2 μc. per g body-weight was injected intraperitoneally at 12 h intervals (Table 1).

From ¾ to 3⅓ h after the last thymidine injection the mice were killed in ether and the thymuses removed immediately.. One half the organ was fixed in 4 per cent neutral buffered formol and embedded in paraffin. Histological sections were Feulgen stained and, after being thoroughly rinsed in distilled water, were coated with '*NTB*$_3$' (Kodak) photographic emulsion in the dark room. Autoradiographs were exposed at 4° C for five weeks and developed, fixed and rinsed in tap water for half an hour, and thereafter in distilled water. The other

Table 1. Labelling Percentage of Thymus Cells after Intraperitoneal Injections of Tritiated Thymidine into *CBA* Male Mice

Mouse No.	Age at 1st inj., days	No. of injections	Dose μc./g	Killed, hours after 1st inj.	Killed, hours after Last inj.	Labelling percentage
1	51	5	1	50	2	45
2	51	5	2	50	2	53
3	51	6	1	63⅓	3⅓	46
4	51	6	2	63⅓	3⅓	54
5	51	7	1	75⅓	3⅓	60
6	51	7	2	75⅓	3⅓	65
7–9*	11	8	2	84¾	¾	88
10–15*	48	8	2	85	1	84

* The thymus glands of mice Nos. 7–9 and 10–15 were pooled.

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half of the thymus was made into a cell suspension. The organ was cut with fine scissors and gently pressed through a stainless-steel mesh into a small volume of Tyrode's solution. For the preparation of smears one part of the cell suspension was mixed with an equal volume of normal mouse serum. A small drop of this mixture was placed on a glass slide which immediately before had been smeared with a solution of 20 per cent mouse serum in 0·25 M sucrose. As the sample of the cell suspension came into contact with the still moist area on the glass slide, it would spread evenly when the slide was tilted slowly. It is believed that this technique for making smears will cause little damage to fragile cells. After drying in cool air, the smears were fixed in absolute methanol for one hour and prepared for autoradiography as described above for the sections. After processing, rinsing and drying, the smears were stained through the 'NTB_3' film with Giemsa stain by the method of Gude *et al.*[9] (Fig. 3). The percentage of labelled cells was determined by counting 1,000 cells. A density of four grains over a cell area corresponding to that of a small lymphocyte was considered as significant labelling. The labelling percentages will be seen in Table 1.

The size of the thymus in the donor animal is also of some importance in the preparation of labelled thymocyte suspensions. To study the changes in size with age, male and female mice were killed at intervals from the day of birth to the age of one year and their thymuses weighed. In male *CBA* mice, the thymus reaches its greatest weight at the age from 20 to 30 days, in the females at the age from 25 to 35 days. The weight of the thymus in relation to body-weight as a function of age

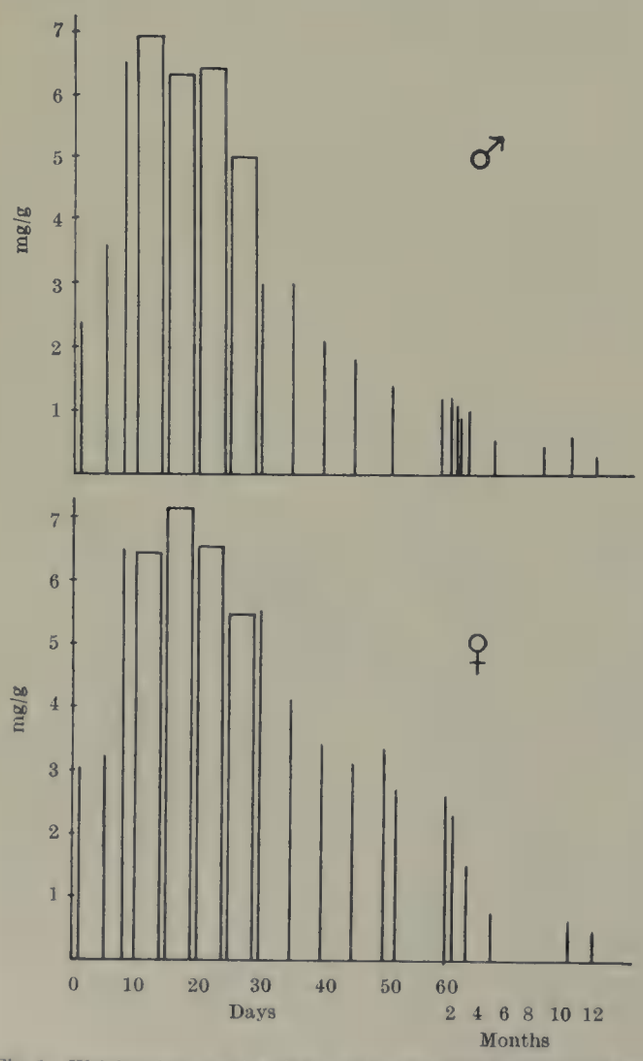

Fig. 1. Weight of thymus in relation to body-weight for male and female mice as a function of age. Each column represents the mean of values from several animals with ages within the interval indicated: days 10–14 from seven mice, days 15–19 from seven mice, days 20–24 from four mice, and days 25–29 from four mice. The single lines represent values for one or the average of two mice from the same day

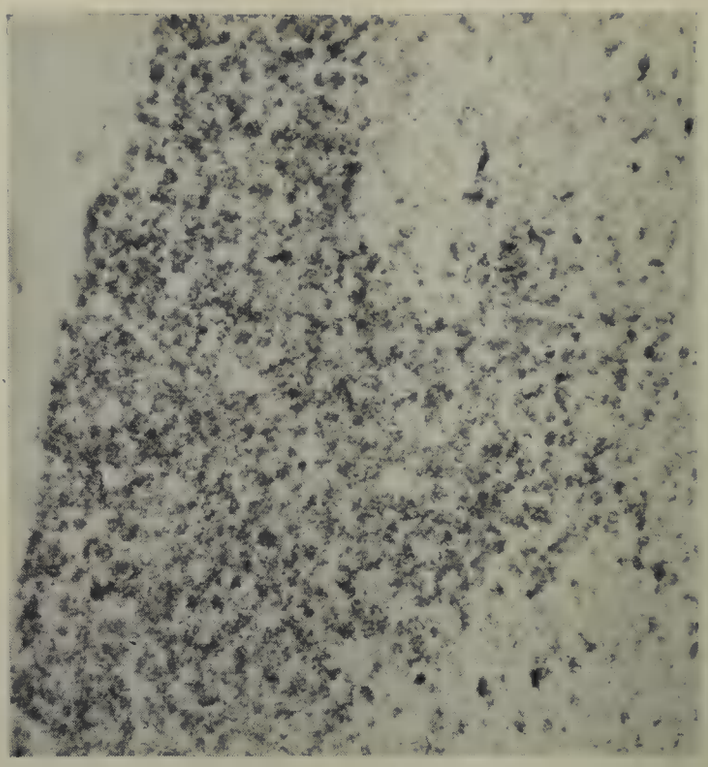

Fig. 2. Autoradiograph of Feulgen-stained section of mouse thymus. The mouse was, at the age of seven weeks, given seven injections of tritiated thymidine intraperitoneally at 12-h intervals. Virtually all cells in the thymic cortex are labelled. (×250)

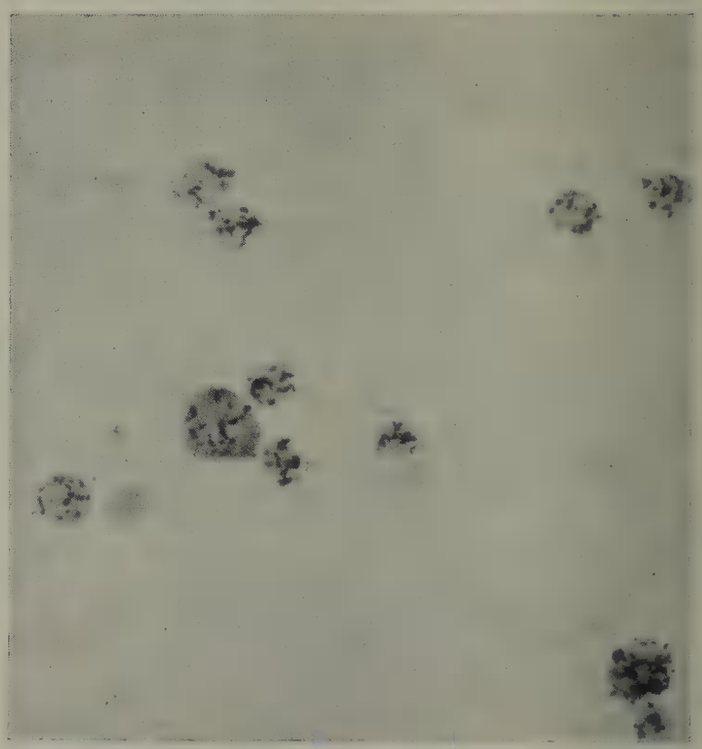

Fig. 3. Autoradiograph of Giemsa-stained smear of a thymus cell suspension. From the eleventh to the fifteenth day of age the mice received eight intraperitoneal injections of tritiated thymidine. All cells except a small lymphocyte carry label. (×1,000)

is shown in Fig. 1. It appears that the relative size reaches a maximum between 10 and 15 days of age in male mice and between 15 and 20 days of age in female mice. As the highest labelling percentage in our study, 88 per cent, was also obtained at the age interval from 11 to 15 days in the males, this age seems most favourable from the point of view of isotope economy in labelling the thymus with tritiated thymidine for the purpose of making cell suspensions.

The high labelling percentage after several injections of tritiated thymidine every twelfth hour is due to the fact that virtually all cells in the thymic cortex carry the label (Fig. 2). In the not so densely populated thymic

medulla the labelled cells are scarce. From autoradiographic studies of cellular kinetics in mouse thymus[10] there is evidence showing that the population of lymphocytes in the thymic cortex is renewed every fourth day. Therefore, for purposes of labelling it seems to be useless to continue injections of tritiated thymidine for longer than four days because of the continuous loss of label from the thymus, probably due to emigration of small thymocytes. Augmenting the dose of tritiated thymidine from 1 to 2 μc. per g body-weight appears to give a higher labelling percentage. It is for the investigator to decide whether the possible radiation damage from the higher dose will be acceptable in his experimental set-up.

Preliminary results from experiments where the donor thymocyte suspension pooled from mice Nos. 7–9 was injected intraperitoneally into isologous mice showed that 1 and 4 days after the injection labelled lymphocytes were found in the spleen and lymph-nodes, and more diffuse labelling was noted in the liver. No labelling was detected in the thymus.

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EFFECT OF A PYRIMIDOPYRIMIDINE DERIVATIVE ON THROMBUS FORMATION IN THE RABBIT

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BORN, Honour and Mitchell[1] showed that the systemic administration of adenosine and 2-chloradenosine modified the behaviour of platelet thrombi in injured arteries in the rabbit. They suggested that if these substances proved to be free from toxic effects, they might be of value in the prevention and treatment of human vascular thrombosis. Adenosine is a powerful vasodilator[2], and produces a marked fall in arterial blood-pressure; moreover, it is rapidly broken down in the body by deamination. 2-Chloradenosine is more resistant to deamination[3], but produced respiratory arrest in two of the rabbits examined by Born, Honour and Mitchell[1]. When Bunag *et al.*[4] reported that a pyrimidopyrimidine derivative (*RA*8-'Persantin'; Boehringer-Ingelheim, Ltd.) retarded the rate of disappearance of exogenous adenosine from whole blood *in vitro*, it seemed possible that this compound might be used to potentiate exogenous adenosine *in vivo*, and permit the anti-thrombotic effect observed by Born, Honour and Mitchell[1] to be obtained with lower doses. A detailed examination of the properties of the compound was therefore undertaken[5]. In concentrations of 10^{-4} M, *RA*8 was found to inhibit the platelet aggregation produced *in vitro* by adenosine diphosphate (ADP); no exogenous adenosine had to be added to produce this effect. It therefore seemed profitable to examine the effects of *RA*8 on thrombus formation *in vivo*, and this article reports the results of these observations.

Grey chinchilla rabbits of either sex (2·9–4·6 kg) were used. Each rabbit was anaesthetized with urethane (6·0 ml. per kg of a 25 per cent solution in 0·9 per cent sodium chloride injected intraperitoneally) and a tracheotomy tube was inserted. Arterial blood-pressure was recorded kymographically from a cannula in the femoral artery and intravenous injections were given through a polythene cannula tied into the left external jugular vein, the catheter being perfused with normal saline at a rate of 0·1 ml./min between injections. Blood samples for platelet clumping were withdrawn from a polythene cannula passed down the left common carotid artery into the aorta. Between samples the cannula was occluded with a wire stilette, and the samples were collected into all-glass syringes, lubricated with silicone fluid (*MS* 550; Hopkin and Williams). Platelet-rich citrated plasma was then prepared, and its clumping activity was determined by the Born optical density method[6], the details of the techniques used being set out fully elsewhere[7].

The right cerebral cortex was exposed and the vessels on its surface observed as described by Honour and Ross Russell[8]. Two degrees of arterial injury[9] were used to assess the effects of *RA*8.

(*a*) *Major injury.* An artery was pinched firmly with needle-pointed ophthalmic forceps, causing bleeding and the formation of a haemostatic plug outside the vessel. Inside the vessel a steady succession of white platelet masses or 'white bodies' formed at the site of injury and embolized. By observing the size of the emboli and by timing their rate and duration of production, the activity of the site could be assessed.

(*b*) *Minor injury.* An artery was gripped gently with the forceps. This did not produce bleeding and no white bodies formed spontaneously. When solutions of ADP, adenosine triphosphate (ATP) or 5-hydroxytryptamine were applied to the external surface of the injury, platelet masses formed in the lumen and embolized for as long as the application was continued. By applying the agents in a standard way (1 drop/sec for 1 min) and by testing progressively lower concentrations, the lowest concentration of each agent which produced white bodies was determined.

(*A*) *RA*8 *given after major injury.* Table 1 summarizes the results obtained in 7 rabbits which were given a single intravenous injection of *RA*8. In each case, a major injury had been inflicted at least 30 min before the injection, and the steady production of white bodies had been

Table 1. Effect of Intravenous Injections of *RA*8 on the Production and Embolization of White Bodies at Major Injury Sites

Rabbit	*RA*8 dose (mg)	*RA*8 dose (mg/kg)	Effect	Interval from injection to effect (min)	Duration of effect
I	40	10·3	White body formation stopped	1	Still present at 5 h
II	10	3·0	,, ,,	9	Present at 4 h
III	10	2·7	,, ,,	2	Present at 3 h
IV	10	2·4	,, ,,	3	Present at 3 h
V	5	1·5	,, ,,	10	Present at 3 h
VI	1	0·3	Small, fragmentary white bodies formed, which washed away as they developed	8	20 min, but masses still abnormal at 4 h
VII	0·25	0·06	Small white bodies which did not stick at bifurcations	5	11 min

observed and timed. When more than 1·5 mg/kg of *RA*8 was given, white body formation was completely abolished, and did not start again during a prolonged period of observation. In these rabbits, a qualitative change in the character of the white bodies was noted in the interval between the injection of *RA*8 and the cessation of activity: the masses became smaller, were pink, floccular and less well defined. They streamed away rapidly from the site and did not become impacted at bifurcations. These changes were similar to those observed by Born *et al.*[1] when adenosine and 2-chloradenosine were given. The lower doses (rabbits VI and VII) did not abolish white body production, but profoundly modified the activity of the site. The white bodies produced were very small, in comparison with those seen in the control period, and for a period of 20 min and 11 min respectively in these two rabbits, the platelet masses consisted merely of swirling fragments which did not stick at bifurcations.

(*B*) *Behaviour of major injuries inflicted after RA*8 *injections*. Major injuries were inflicted on two rabbits, and observed for 15 min, during which period a steady succession of dense white bodies formed and embolized. When these masses left the injury site they stuck at bifurcations. Each rabbit was given 10 mg *RA*8 intravenously and in 2 and 3 min respectively the initial injury sites were inactive. Major injuries were then inflicted at intervals after the *RA*8 injection, new arterial systems being used for each injury. Table 2 shows that the duration of white body formation at each new injury site increased with the passage of time after *RA*8 administration. The nature of the masses produced also showed a graded response with time: injuries inflicted immediately after *RA*8 could only produce a faint hazy mass, while at longer intervals after the injection the masses became larger, more persistent and more dense. None of the injuries produced an unusual amount of bleeding, and firm external haemostatic plugs formed on the outside of the injured vessels in the usual way. Even the injuries inflicted immediately after the *RA*8 had been given showed a normal haemostatic response, although these sites could only produce feeble, transient white bodies. Born *et al.*[1] found a similar dissociation between haemostasis and thrombosis when adenosine and 2-chloradenosine were given, and this holds out the hope that effective antithrombotic drugs may be found which do not prejudice haemostasis. The dissociation between external and internal platelet plug formation may mean that the stimulus to platelet aggregation in blood shed from a vessel is different from the mechanism which operates within the lumen, or that the mechanism is the same, but that the initial response of the injury site is so intense that it cannot be blocked by any of the agents so far tested.

(*C*) *Effect of RA*8 *on minor injuries*. Table 3 shows the effect of *RA*8, 10 mg given intravenously, on the lowest concentrations of the three clumping agents which were able to produce white bodies when they were applied to a minor injury site. Eighteen min after *RA*8 had been given, the response to 5HT had been abolished, while the end-point levels for ADP and ATP had only changed by a factor of two and four respectively. However, the character of the ADP and ATP response had been profoundly changed. Under control conditions, increasing the concentration of the locally applied substances above the end-point produces progressively larger, denser and more persistent bodies; after *RA*8 this graded response was lost and ADP and ATP at concentrations of up to 250 μg/ml. produced the same faint, transient masses which the end-point concentrations evoked. 5 h after the *RA*8 injection the site still did not respond to 5HT, 250 μg/ml., whereas it had regained its responsiveness to ADP and ATP, the end-points having returned to their pre-*RA*8 levels and the normal gradation of response being restored.

Table 2. Duration of White Body Formation at Major Injuries inflicted at Various Intervals after a Single Intravenous Injection of *RA*8

Rabbit	*RA*8 dose (mg)	*RA*8 dose (mg/kg)	Interval between *RA*8 injection and injury (min)	Duration of white body formation (min)
VIII	10	2·6	2	2
			15	4
			40	7
			80	18
IX	10	2·7	3	3
			17	10
			32	15
			60	35
			107	37

Table 3. Effect of Intravenous *RA*8 (10 mg, 2·6 mg/kg) on the Minimum Effective Concentrations of ADP, ATP and 5HT which were capable of producing White Bodies at a Minor Injury Site

Substance	Minimal effective concentration (μg/ml.) Before *RA*8	18 min after *RA*8	At 30-min intervals after *RA*8	5 h after *RA*8
ADP	8	16	Not tested	8
ATP	2	8	Not tested	2
5HT	2	No effect from 250	No effect from 250	No effect from 250

Table 4. Effect of a Series of Injections of *RA*8 on the Minimal Concentration of 5HT which was capable of producing White Bodies at a Minor Injury Site

*RA*8 dose (mg)	*RA*8 dose (mg/kg)	Minimal effective concentration of 5HT (μg/ml.) at specified intervals after *RA*8 injections			
0	0		0·5		
0·25	0·09	+6 min	2·0	+60 min	2·0
0·5	0·17	+3 min	8·0	+37 min	8·0
1·0	0·34	+2 min	64·0	+60 min	4·0

Table 4 shows that the effect of *RA*8 on the 5HT sensitivity of a minor injury site is closely related to the dosage used, 0·25 mg giving a four-fold decrease in sensitivity, 0·5 mg a sixteen-fold reduction and 1·0 mg reducing it by a factor of 1/128. In rabbit X (Table 3), which received 10 mg *RA*8, the change in 5HT response persisted for more than 5 h, whereas in rabbit XI, which received only 1·75 mg, spread over 97 min, the 5HT responsiveness was beginning to return some 60 min after the last *RA*8 injection.

(*D*) *Effect of local application of RA*8. A major injury was inflicted, and was observed to produce dense, adherent white bodies for 23 min. *RA*8, 500 μg/ml. in physiological saline, was then applied to the external surface of the injury at 1 ml./min for 5 min. As soon as the application began, the white bodies became smaller (only growing half-way across the lumen before embolizing, whereas they had previously been filling the whole lumen) and were less dense, appearing to wash away as rapidly as they were forming. Twenty-one min after the beginning of the application, the site became inactive and did not regain its activity in a 200-min period of observation. 107 min after the application, the responsiveness of the site to ADP, ATP and 5HT was tested. ADP and ATP both produced characteristic white bodies (end-points 2 μg/ml. for each agent with a normal gradation of response to higher concentrations) while 32 μg/ml. of 5HT was needed for white body production. Higher concentrations of 5HT produced white bodies similar in size, density and duration to those seen with the end-point concentration. A second major injury was then inflicted, and after it had produced white bodies for 15 min, *RA*8, 500 μg/ml., was applied as already described here. Once again, there was immediate reduction in the size of the white bodies, and 20 min after the application, the site became inactive.

A major injury was inflicted on another rabbit. The site produced huge, tenacious white masses for 26 min. *RA*8, 100 μg/ml. in saline, was then applied, and within 1 min the white mass present at the site began to break up and wash away. Small, fragmentary white bodies continued to form, but 37 min after the start of the application the site became quiescent and remained so for the remaining 2 h in which the preparation was observed.

(*E*) *Effect of RA*8 *on circulating platelets*. The inhibitory effect of *RA*8 applied locally to injured arteries shows that the agent can act directly on the injury site, but in view of the reduction of *in vitro* platelet clumping activity observed by Emmons *et al.*[5] when *RA*8 was added to platelet-rich plasma systems, we measured the clumping

activity of rabbit platelet-rich plasma before and at intervals after an injection of *RA*8. Two samples of arterial blood were obtained, and *RA*8, 10 mg, was then given intravenously. Further arterial blood samples were obtained 2, 10–19, 20–40 and 60–90 min after the injection. Platelet-rich citrated plasma was prepared, and its clumping response to ADP 1·5 μg/ml. was measured. For each sample the maximum fall in optical density observed after the addition of ADP was expressed as a percentage of the fall in optical density produced by high-speed centrifugation. The mean percentage fall in optical density for the two control samples was then taken as unity, and the clumping activity at the various times after *RA*8 was expressed as a ratio of this control level. The *RA*8 content of platelet-poor plasma was measured spectrofluorimetrically as described by Beisenherz *et al.*[10]. The results are shown in Table 5. There was a marked reduction in ADP-induced clumping 2 min after the injection of *RA*8; in the next sampling period this inhibition was less marked, and thereafter platelet-clumping activity had returned to its control-level.

Table 5. Mean Clumping Activity with ADP 1·5 μg/ml. expressed as Ratio of Clumping Activity in Control Period, and Mean Plasma *RA*8 Levels, in 6 Rabbits given 10 mg *RA*8 intravenously

Time (min) after *RA*8 injection	Clumping ratio	Plasma *RA*8 level (μg/ml.)
2	0·76	3·05
10–19	0·88	0·82
20–39	0·99	0·48
60–89	0·95	—

Our results show that *RA*8 profoundly modifies thrombus formation in injured arteries. The efficacy of topical applications, and our inability to demonstrate anything more than a transient change in the clumping activity of the circulating platelets, suggest that the agent is acting on the site of injury rather than on the circulating blood. Honour and Mitchell[9] showed that thrombus formation in damaged vessels is brought about by compounds similar in properties to ADP and ATP. These are formed in the injured tissues, and are released into the lumen. *RA*8 must interfere with this mechanism, either by cutting off the supply of thrombus-producing material or by producing an inhibitory substance.

*RA*8 has been shown to possess many interesting properties; it has marked vasodilator activity, produces some reduction in blood pressure, increases the rate and depth of respiration and produces bronchodilatation[11]. The sites remain responsive to infused catecholamines, so simple sympathetic blockage cannot account for these changes, and Unghvary *et al.*[12] have produced an alternative mechanism by showing that the agent produces a reduction in circulating catecholamines. *RA*8 has other more fundamental actions, for it increases glucose consumption and reduces lactic acid formation in the hypoxic heart[13]. This finding may relate to the work of Frimmer *et al.*[14], who showed that *RA*8 was fixed by mitochondria and that it reduced the critical oxygen pressure in tissue culture studies. In ischaemic heart muscle, *RA*8 increases the adenosine content; this increase results from decreased adenosine breakdown, rather than increased formation[15]. *RA*8 might therefore interfere with white body formation in our injured arteries by allowing adenosine to accumulate at the injury site. However, topical and systemic *RA*8 produce immediate inhibition, whereas there is a lag of some 30 min after systemic adenosine administration before an inhibitory effect is seen, while topical adenosine is inactive. It therefore seems probable that *RA*8 interferes directly with the formation by the injury site of the white-body producing substance. Alternatively, it may affect the way in which the thrombus-forming message is propagated through the growing platelet mass. After injury, the first layer of platelets to adhere to the injury site must be capable of transmitting information to oncoming cells, and this process continues until the mass completely blocks the lumen. *RA*8 might be acting on the circulating cells, and rendering them incapable of propagating the injury message. The methods of studying platelets described here may not be capable of detecting this change. It would be of interest to know whether a major injury site rendered inactive with *RA*8 is free from platelets, because the site is no longer producing the injury substance, or whether it is covered with a thin layer of platelets which are not propagating the injury message. We hope that electron micrograph investigation will help to resolve this question.

As *RA*8 exerts such a powerful anti-thrombotic effect in injured arteries, could it be of value in the management of human vascular thrombotic disease? Mitchell[16] has suggested that there are two alternative models of thrombus formation. In the first, hitherto normal circulating cells encounter an abnormal arterial segment. This is the situation in the injured rabbit cortical arteries which we have used to investigate *RA*8. In the second system, the circulating cells are already abnormal and are ready to adhere together; the location of the thrombus is determined, not by a message from an abnormal vessel wall, but by critical flow situations which permit cell collision and adhesion. We do not know which of these model situations is relevant to human disease, but would point out that our studies on the efficacy of *RA*8 only pertain to thrombus formation in injured vessels, and we need further information on the action of *RA*8 in man.

We have examined the effect of intravenous and oral *RA*8 on the behaviour of human blood platelets[5], and have shown that spontaneous platelet aggregation is reduced. Further examination of the clinical effects of *RA*8 in thrombotic disease is clearly called for; unlike adenosine and 2-chloradenosine, which would need detailed toxicity investigations before they could be used therapeutically, there is already a substantial corpus of information available about *RA*8. It appears to be remarkably non-toxic for a drug with such varied and powerful actions. Kadatz[11] showed that in mice the acute LD_{50} for intravenous *RA*8 was 150 mg/kg, while for oral administration it was 2,150 mg/kg. In long-term investigations, dogs were given 20 mg/kg/day, and rats received 100 mg/kg/day, without ill effect. We have used 600 mg/day by mouth in man[5] obtaining plasma *RA*8-levels of up to 5 μg/ml. (10^{-5} M) without ill effect. One undesirable property of *RA*8 might be its hypotensive effect; but in our anaesthetized rabbits we did not observe a reduction in pressure of more than 30 mm mercury. This is in accordance with the experience of Kadatz and Beisenherz[17], who found a similar fall in blood pressure in dogs given 1·0 mg/kg (18·5 mm pressure reduction) to those given 4·0 mg/kg (23·8 mm pressure fall). We would therefore suggest that, in addition to the contribution made by *RA*8 to the investigation of experimental thrombus formation, its lack of toxicity makes it a suitable material for clinical investigations in man.

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A SEARCH FOR MUTATION IN CHEMICAL SYSTEMS AT STEADY STATE

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IN a steady state, a transient condition is maintained by continuous repetition. A complex system (for example, an organism) may be essentially in a steady state with respect to all major components, while minor components continue to change. A chemical mixture containing active reagents and continually replaced at a slow rate with freshly mixed solution approaches a steady state gradually. In such an 'open' system, systematic changes will presumably become smaller and smaller until they reach the level of random molecular events. A late, small systematic change and even a single molecular event might be detectable if amplified in some way.

Catalysis and especially autocatalysis seem to offer promise as amplifiers. In an open chemical system, a late-appearing minor product which inhibited or catalysed some early and major reaction might alter the number or proportions of the recognized components. Such a catalyst, interesting in itself, could be inferred from its effects on the system even if it was below the minimum concentration for direct assay. Thus, if one cannot deliberately use catalysis to detect late changes in an open system, one may investigate late changes in the hope of detecting interesting examples of catalysis.

With respect to molecular species having individually small probabilities of occurrence, the mixture would continue to change indefinitely. The more probable molecules would appear early and frequently; the least probable molecules, only singly and at long intervals. One of these rare molecules, even if it were a powerful catalyst, could not exert enough effect on other reactions to be detected during its lifetime. However, such a molecule might catalyse the formation of one of its own precursors. If in its lifetime the first molecule of catalyst increased the amount of precursor enough to make probable the formerly improbable synthesis of catalyst molecules, an autocatalytic system would result. The precursor and all derivatives including the catalyst would tend to increase until side reactions or the reverse of the catalysed reaction imposed a new steady state. If the reactants in the catalysed step were sufficiently abundant, one or more of the derivatives could reach detectable levels so that the new mixture would be recognizably different from the original.

This phenomenon, a late change due to the origin of a self-catalysing system, will be referred to as mutation in the common non-technical sense of the word and also in analogy with genetic mutations in organisms. Such a mutation can be defined operationally as a permanent change of composition in an established steady state such that the new composition can be induced in another similar mixture by a small inoculum from the original. It is not important whether the catalyst was present at very low concentration in the first steady state, or whether it appeared after an interval as a single, improbable molecule; these conditions are interchangeable with changes in volume and molar content of the system.

The general interest attached to homogeneous catalysis by organic molecules and particularly to simple self-reproducing systems[1–3] prompted the search described here. Despite negative results, it is reported as an investigation of evolution in a chemical system[4], distinct from the already frequent and successful investigations of processes which could initiate chemical evolution[5]. Since there would be little reason for others to repeat the negative experiments, the findings and methods will not be described in complete detail. It is appropriate, however, to report the number and diversity of experiments and the analytical effort which will have to be improved if the demonstration of mutations in chemical systems is to succeed.

To maximize the number and rate of reactions and the likelihood of catalytic effects, it seems appropriate to use highly reactive compounds at moderately elevated temperatures and in maximal concentration consistent with a homogeneous solution. An approximate steady state can be maintained by periodic renewal of part of the mixture. Recognition of late changes which yielded new products would require only standardized analysis and detection, not identification of components. One or more new spots appearing during chromatography after a solution had apparently stabilized, or a sharp change in relative intensity of certain spots, would direct attention to possible catalysis by a late product. A mutation would then be indicated if the old and new chromatographic results could be produced side-by-side in simultaneous analyses of two freshly-started solutions, one of which had been 'seeded' with the old solution. Even if no change had been detected in the original steady state, a reproducible difference between seeded and unseeded solutions would demonstrate an effect of inoculation that might represent catalysis.

The experiments were conducted in 100-ml. samples in side-arm flasks half immersed in oil at 90° C. The flasks were connected through reflux condensers to a slowly flowing stream of nitrogen under slight positive pressure. Most of the seeding tests were conducted in smaller samples in side-arm test-tubes with cold-finger condensers. The basic reagents, formaldehyde and cyanide, were selected as the most reactive one-carbon compounds readily obtainable in a high state of purity. Ammonia, allyl alcohol, diacetyl and pyruvic acid were added singly or in combination to some solutions, and in two solutions cyanide was omitted. Each of nine combinations was replicated in solutions buffered at *p*H 7 and *p*H 9 with sodium, chloride and phosphate ions. Table 1 shows the main reagents in the eighteen experiments.

Experiments 17 and 18 were initiated just two months before the end of the project, but the other experiments ran for between 14 and 20 months. The initial renewal schedule was 10 per cent daily, and after 2–4 months the interval was lengthened to 20 per cent semi-weekly or 10 per cent weekly in different experiments. A number of the solutions were eventually modified by addition of minerals in catalytic quantities: $MgSO_4$, $CaCl_2$, $CuSO_4$, $FeSO_4$, $MnCl_2$, $ZnCl_2$, KI, I_2, and reduced sulphur as cysteine. Continuous ultra-violet radiation[3] was supplied in experiments 17 and 18 and in the last four months of experiments 13–16 by means of pencil-shaped mercury vapour lamps mounted in the side-arm stoppers and dipping under the surface of the solutions.

Samples were analysed by evaporation to dryness under nitrogen at 40° C, and the initial distillate was collected

Table 1. Molar Concentrations of Principal Reagents

Odd-numbered solutions were buffered at *p*H 9; even-numbered solutions at *p*H 7

	Exp. (solutions)								
	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18
Sodium cyanide	0·1	0·1	0·3	0·3	0·3	0·3	0·3	0·1	0
Formaldehyde	0·1	0·1	0·3	0·3	0·3	0·3	0·3	0·1	0·1
Ammonium hydroxide		0·1		0·3	0·3	0·3	0·3	0·1	0·1
Allyl alcohol					0·1			0·1	0·1
Diacetyl						0·1		0·1	0·1
Pyruvic acid							0·1	0·1	0·1

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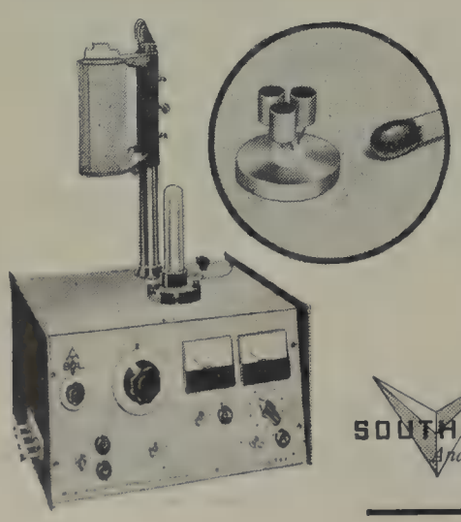

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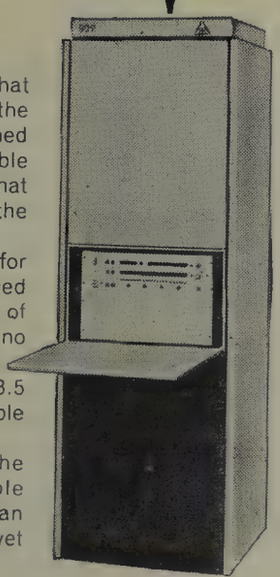

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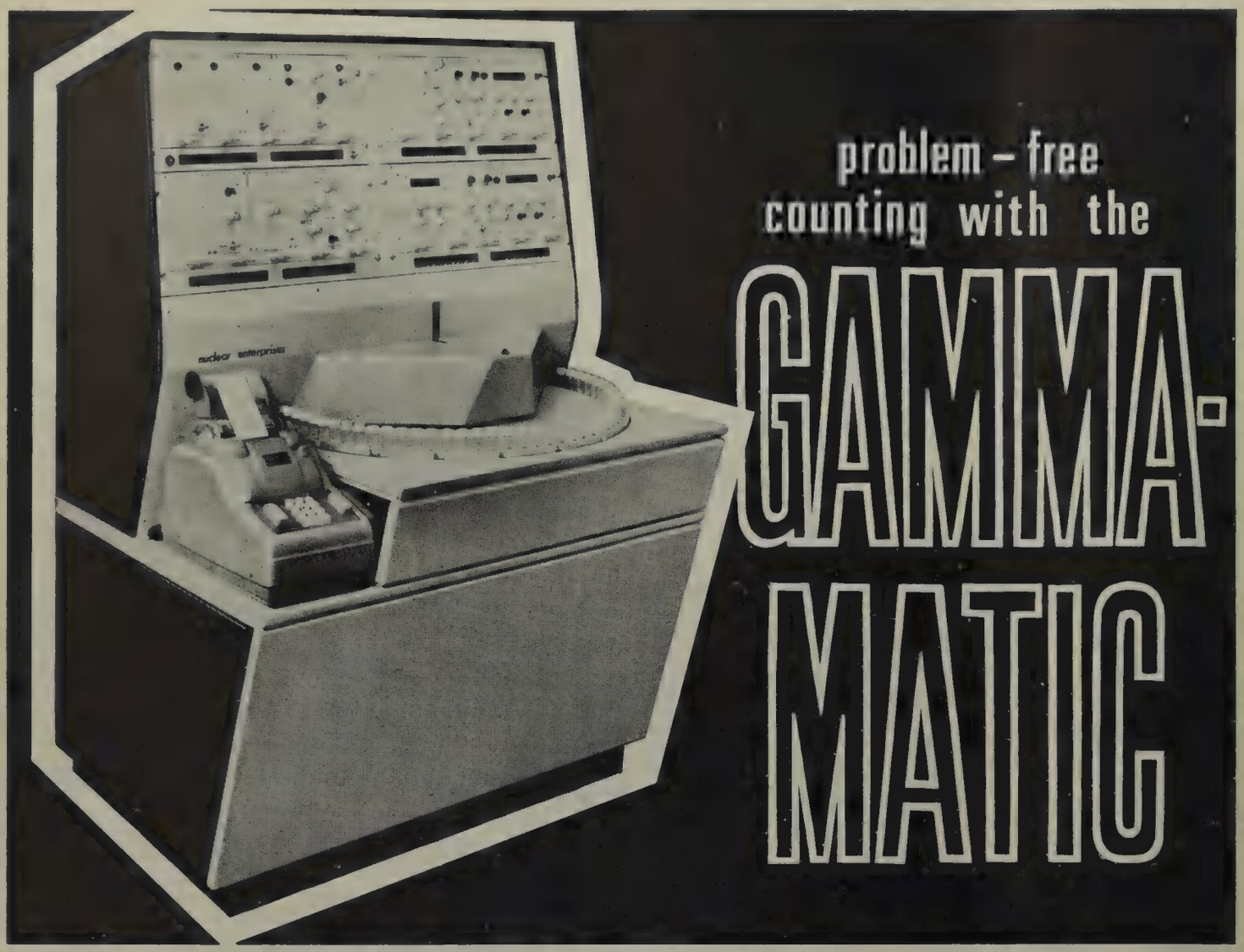

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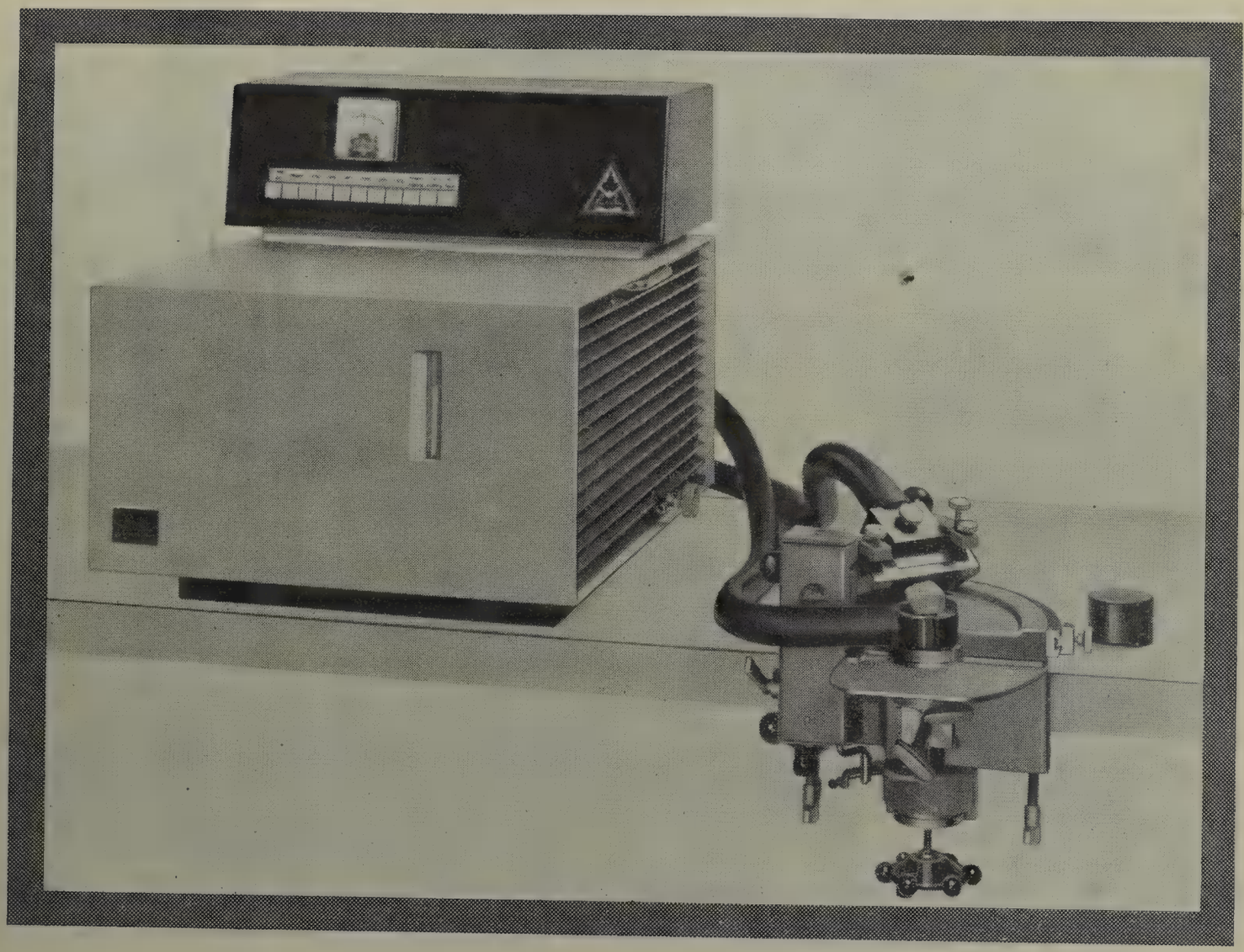

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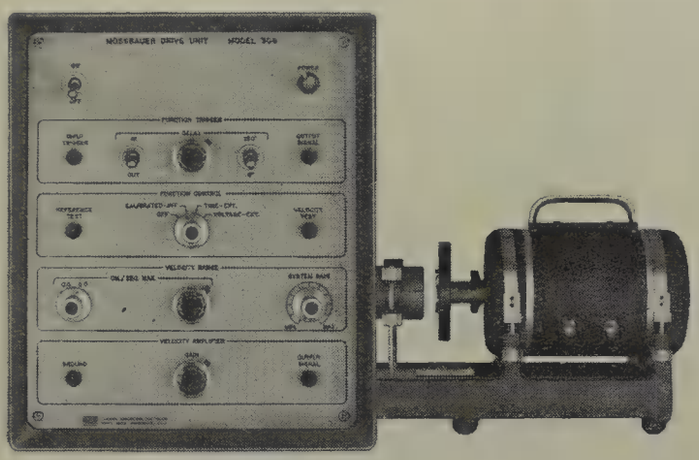

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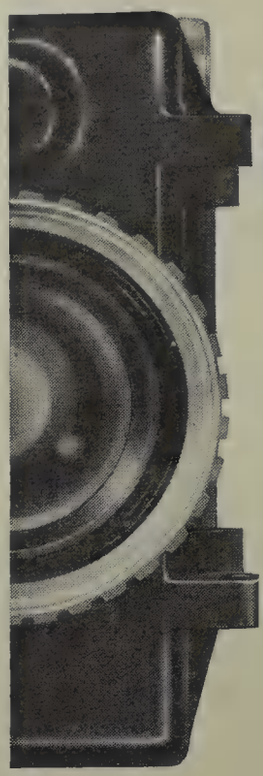

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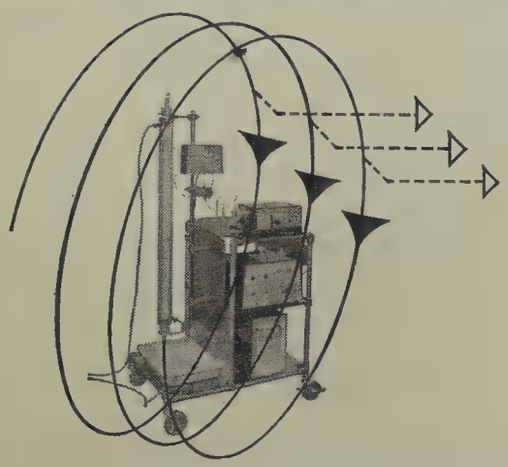

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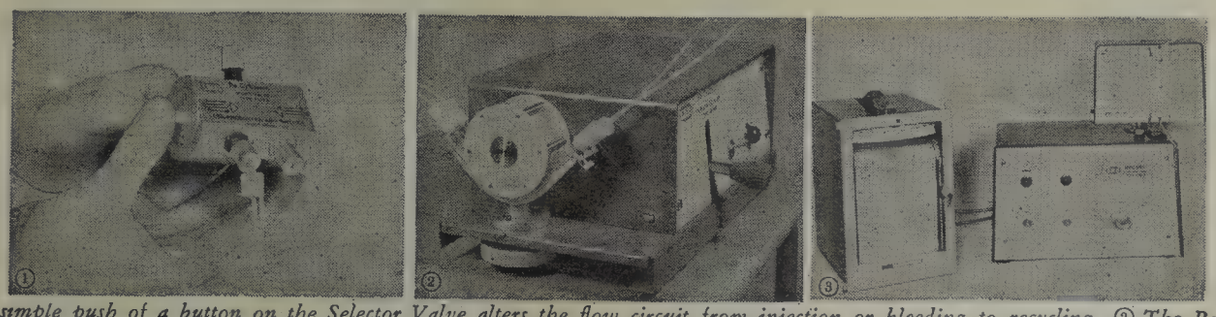

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for gas chromatographic analysis. The residue after distillation was extracted in turn with methyl acetate and methanol and was finally re-dissolved in water. The three resulting extracts were chromatographed in two dimensions on thin layers of silica gel. Three pairs of solvent mixtures were devised to give good separations of each extract in the early experiments (Fig. 1), but for the more complicated solutions these systems were supplemented with one-dimensional chromatography in other solvents (Fig. 2). The dry chromatograms were in most cases sprayed with alkaline $KMnO_4$, yellow spots on the pink background were marked to indicate their order of appearance, and the results were recorded as negative photographic prints before they began to fade. In most of the seeding tests, carbon-14-labelled formaldehyde and/or cyanide were used and autoradiograms were made[6]. Some chromatograms, particularly in the seeding tests, were examined by means of more sensitive or more specific location reagents or were viewed under ultra-violet light.

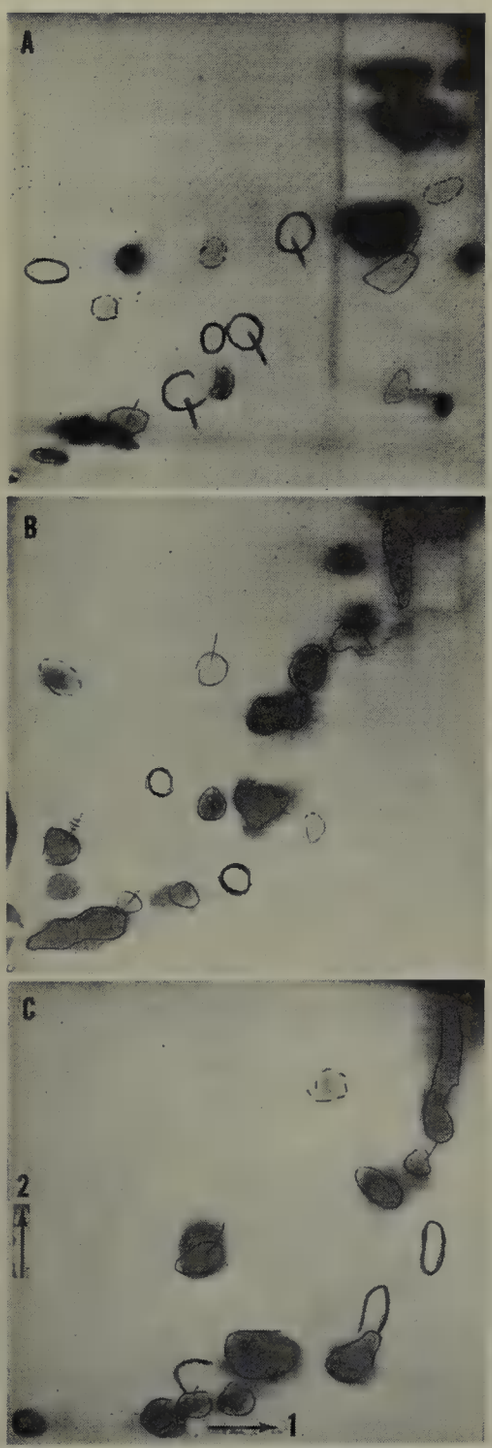

Fig. 1. Typical thin-layer chromatograms of solution 3 in the steady state. *A*, Methyl acetate extract; *B*, methanol extract; *C*, extracted residue. Each fraction was chromatographed with a different, appropriate pair of solvents, but there is some duplication of separated compounds in successive chromatograms

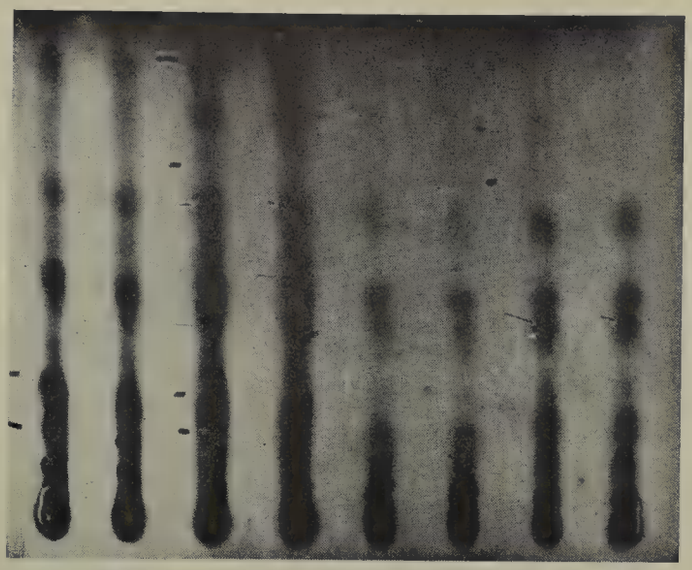

Fig. 2. Methyl acetate extracts chromatographed in a water-free system to separate compounds that coalesced near the front in the usual two-dimensional analysis (compare with Fig. 1 *A*). Extracts of four pairs of solutions obtained in seeding tests of experiments 15, 16, 17, 18; the left member of each pair had been seeded

Hydrogen cyanide in aqueous ammonia is known to produce a large variety of biologically interesting compounds spontaneously. Oro[7] identified seven compounds inlcuding adenine. At least 75 ninhydrin-reacting compounds have been distinguished after hydrolysis of the products and at least fifty compounds absorb or fluoresce in ultra-violet light[8]. The reactions of alkaline cyanide with formaldehyde also proceed rapidly and yield a variety of compounds[9]. In the experiments recorded here, cyanide and formaldehyde yielded 27 reproducible spots on chromatograms of the entirely non-overlapping aqueous and methyl acetate extracts when permanganate was the location reagent. Autoradiography revealed as many as 48, and many additional spots could be detected by fluorescence. Four strong spots were ninhydrin positive and many others revealed nitrogenous content by reacting with sodium hypochlorite or tertiary butyl hypochlorite. A few spots gave one or more positive tests for double bonds, phenols, esters and aldehydes. Gas–liquid chromatography of the formaldehyde–cyanide mixtures in a steady state revealed from three to five products; but the method would not have detected highly polar compounds.

Addition of other reagents resulted in more numerous products. Thus, gas chromatograms of solutions 15 through 18 all had at least 19 indicated peaks in addition to those obtained with the reagent blanks (Fig. 3). Solutions containing diacetyl acquired a surface film and a variable amount of brown turbidity or sediment. Changes in renewal schedule and addition of minerals had surprisingly little effect on most of the chromatograms. Ultra-violet light had little effect in the turbid solutions, but changed solutions 13 and 14 rather extensively. In addition to the regularly appearing spots, others occurred in only a few analyses or were poorly differentiated. Some which appeared late in the investigation could be attributed to improvements in technique or to changes in the experiments. While the less-reliable spots could not be taken as evidence of mutation in themselves, they could be examined to advantage in the seeding tests, in which seeded and control solutions were analysed simultaneously and compared in fine detail (Fig. 2).

The reactions proceeded rapidly in all solutions, yielding yellow or red-brown colour in the first hour and going on to nearly black in many of the mixtures. Nearly all spots which appear on chromatograms could be found at 24 h, and after four days there were only minor changes. In experiments 1–8, control solutions were maintained without renewal for several weeks; they yielded pro-

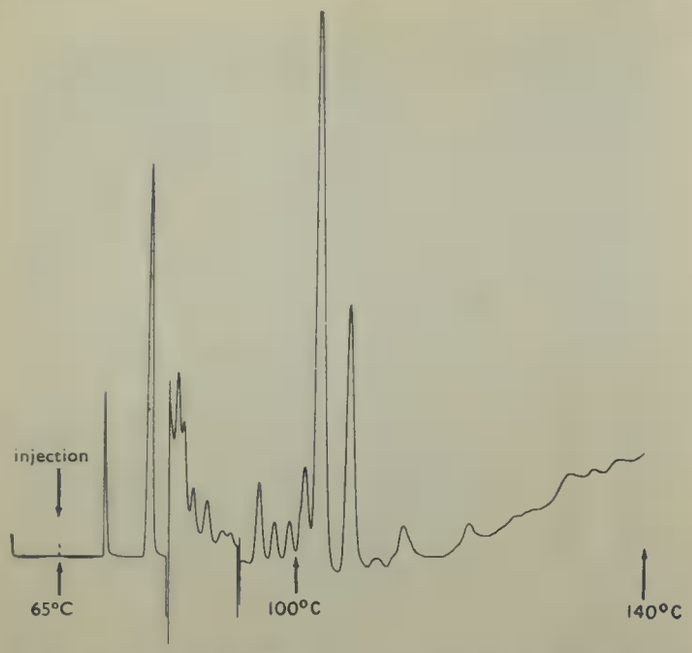

Fig. 3. Gas–liquid chromatogram of volatile fraction, solution 17. The two deflexions below the base-line correspond to increases in sensitivity by factors of 200 and 2, respectively. The second peak coincides with that of one reagent, allyl alcohol. Column used: 'Castorwax' on 'Chromosorb *W*' (*HMDS*) (Wilkens Instrument Co.), 2 meters

gressively simpler chromatograms, and alkaline solutions 1 and 5 lost colour.

No good evidence of mutation was found. A few differences between seeded and control solutions were observed, but these were either not reproducible or could be attributed to late reaction products in the seeding solutions, detectable at the effective dilution of about 1 : 20. The only observed late change in a stable mixture was seen in the physical characteristics of solution 1. After about five months this solution became relatively resistant to evaporation in the analytical procedure. This change did not coincide with any detectable change in the chromatograms; it was not perpetuated in seeding tests and disappeared from the flask within three weeks.

Failure to demonstrate mutations in these experiments does not, of course, prove that mutations cannot occur. The variety of reagents and conditions was limited and detection of products was not exhaustive even at concentrations of more than 10^{16} molecules in 100 ml. However, the total number of molecular species produced and given an opportunity to catalyse ongoing synthesis of their own precursors was probably many hundred. In addition, contamination by micro-organisms must have added innumerable compounds of known biological activity. The reaction conditions were intentionally inimical to reproduction by whole organisms and would have quickly degraded their enzymes, but some native compounds and many modified ones would have been stable. If any of these contaminating compounds could have been produced in one or more of the solutions, they had an opportunity to catalyse their own synthesis or that of their precursors.

It is possible that similar experiments with different reagents might yield detectable mutations; in that event, the required catalysis may depend on rather special reagents and conditions, and is not a frequent phenomenon at low levels of molecular complexity. It is also possible that some of the molecules occurring in these experiments did catalyse steps in their own synthesis, but that inefficiency of the catalysis or slow intervening steps prevented detectable change. Several such mutations would have to accumulate and provide a more or less complete 'metabolic' system[1] before any catalysts could reach concentrations that would alter the major components. If such a low level of efficiency is characteristic, mutations can be detected only in more prolonged experiments, by more sensitive analytic techniques, or by the application of quantitative and statistical methods.

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PLAQUE-FORMATION BY NON-IMMUNE AND X-IRRADIATED LYMPHOID CELLS ON MONOLAYERS OF MOUSE EMBRYO CELLS

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THE demonstration that immune lymphoid cells are capable of killing histo-incompatible target cells *in vitro* has greatly facilitated the examination of the mechanism of cellular immunity in tissue transplantation[1,2]. Various characteristics of the cytotoxic effect of humoral antibodies and sensitized cells on different targets appear to justify the conclusion that they act by different mechanisms. Thus, cytotoxicity caused by cellular immunity does not require the participation of complement in contrast to the action of humoral isoantibodies. Furthermore, target cell death was detected after 24–48 h, subsequent to treatment with immune lymphoid cells, whereas humoral antibodies killed within 1 h in the presence of complement[2]. As a matter of fact, an antagonistic relationship exists between cellular and humoral immunity in tissue culture and *in vivo* as well[3,4]: pretreatment of neoplastic target cells with humoral isoantibodies inhibited the cytotoxic effect of immune lymphoid cells partly or completely, presumably by competition for the same antigenic determinants.

A fundamental difference was revealed between cellular and humoral immunity by the finding that target cell death subsequent to treatment with immune lymphoid cells could be ascribed to close contact with the foreign histocompatibility antigens carried by the lymphoid cells[2]. It could be shown that normal allogenic lymphoid cells were as efficient in killing target tumour cells *in vitro* as immune cells, provided that they were aggregated to the targets by phytohaemagglutinin or heterologous antibodies; aggregation of isogenic lymphoid cells had no detectable effect. The cytotoxic action of non-immune lymphoid cells was not caused by the induction of a primary immune response *in vitro* since semi-isogenic F_1 hybrid lymphoid cells killed the parental target cells efficiently, although they were genetically incompetent to respond immunologically[2]. The

results suggested that immune lymphoid cells were equipped with a specific receptor making them capable of becoming attached to the target cells; target cell death was not due to an immunological reaction, however, but appeared to be related to a non-immunological recognition process of antigenic and/or structural incompatibility, leading to cell death by so far unknown reactions. This phenomenon has been termed *antigenic recognition*.

In the work recorded here a plaque-technique was used to evaluate the effect of normal lymphoid cells aggregated to monolayers of embryonic target cells. Particular interest has been focused on the importance of genetic disparity between the non-immune lymphoid cells and the embryonic targets.

Monolayers of mouse embryo cells were prepared by treating fragments of embryos (14–19 days old) with 0·25 per cent trypsin for 1 h at room temperature. The cells were washed and suspended in lactalbumin in Earle's solution supplemented with 10 per cent foetal calf serum. 10^7 Trypan blue unstained cells were added to each 10 cm diameter Petri dish and 3×10^6 cells to each dish with 5 cm diameter. After 24–48 h inoculation the medium was replaced with Parker's Medium 199 supplemented with 10 per cent calf serum and containing phytohaemagglutinin diluted 1/50–1/100. One hour later 0·1 ml. of a lymphoid cell suspension containing $5–10 \times 10^6$ cells was carefully dropped on to a limited area of the monolayer culture. The lymphoid cells usually occupied an area with a diameter of 1–2 cm, but did not spread further and became firmly fixed to the monolayer culture due to the presence of phytohaemagglutinin. Each Petri dish received lymphoid cells at 3–4 different spots, one suspension always containing isogeneic cells.

In some experiments the lymphoid cells were irradiated with 1,500 r. 1–2 h prior to the addition on to the monolayers. Irradiation was carried out with cell suspensions in Petri dishes. The dose of irradiation was sufficient to prevent the division of the lymphoid cells completely as shown by their failure to incorporate thymidine labelled with carbon-14 subsequent to stimulation with phytohaemagglutinin. In contrast a marked stimulation of isotope incorporation was observed with non-irradiated cells after 24 h.

The effect of aggregation of non-immune lymphoid cells on embryonic target cells was evaluated by the appearance of plaques in the monolayer culture at the site of lymphoid cell introduction (Fig. 1). An analogous technique has been described by Granger and Weiser[5] for the detection of cytotoxic effects mediated by immune macrophages on monolayer cultures of neoplastic cells. In our system the plaques were seen macroscopically as a clear circular area in the monolayer (Fig. 1). Microscopically the plaques were found to contain a few scattered embryo cells, usually surrounded by aggregated lymphoid cells (Fig. 2). In cases where no plaques developed the monolayer cells were intact and surrounded by dense aggregates of lymphoid cells.

The addition of allogenic or semi-isologous F_1 hybrid spleen or lymph node cells on to monolayers of parental embryo cells in several different strain combinations led to the appearance of plaques, whereas isogenic lymphoid cells had no detectable effect (Table 1, Exps. 1–7). The plaques appeared at the same time in each experiment, but the time needed for detectable plaque formation varied between 1 and 6 days in different experiments. In the majority of the experiments the cultures were observed for 4–7 days after the first appearance of the plaques, but no further plaques were found to appear. It cannot be stated with certainty that plaque formation was caused by target cell death, but the results are in agreement with the earlier findings, demonstrating that both F_1 and allogenic cells were capable of killing parental neoplastic target cells *in vitro*. The previous investigations led to the conclusion that close contact with foreign histocompatibility antigens resulted in the target cell death. It was of considerable interest, therefore, to study the effect of parental lymphoid cells on F_1 hybrid targets, since the parental lymphoid cells are not expected to contain any antigens foreign to the target cells.

Non-immune parental lymph node or spleen cells were found to be fully competent to induce the appearance of plaques in the majority of cases studied (Table 1, Exps. 8–13). Thus, parental lymphoid cells behaved as allogenic cells in this respect. However, exceptional results were obtained with some parental lymphoid cells. Thus, *CBA*, *C3H* and *DBA/2* cells failed to induce plaques on hybrids with strain *A* as the other partner, although both non-immune *A* parental lymphoid cells and allogenic cells were efficient. It is not possible by the technique to differentiate between a complete non-reactivity and a decreased efficiency of the lymphoid cells. However, recent findings[5] obtained with a quantitative test system described previously[2,3] showed that non-immune parental lymphoid cells of the genotypes mentioned killed efficiently semi-isogenic F_1 hybrid targets. It seems possible, therefore, that the present results are due to quantitative factors. The failure of *CBA*, *C3H* and *DBA* cells to affect the hybrid targets was not limited to hybrids with strain *A* as the other partner, but was also with $(A.SW \times CBA)F_1$ hybrids and with allogenic (*5M*) target cells. A discrepancy between the effect of two parental cells on hybrid targets was also noticed in one experiment with *A* and *C57BL*

Fig. 1. Macroscopic appearance of plaques on $(A \times C57BL)F_1$ mouse embryo cells. At the indicated sites were added $(A \times C57BL)F_1$ (1), *A* (2) and *A.CA* (3) X-irradiated (1,500 r.) lymphoid cells

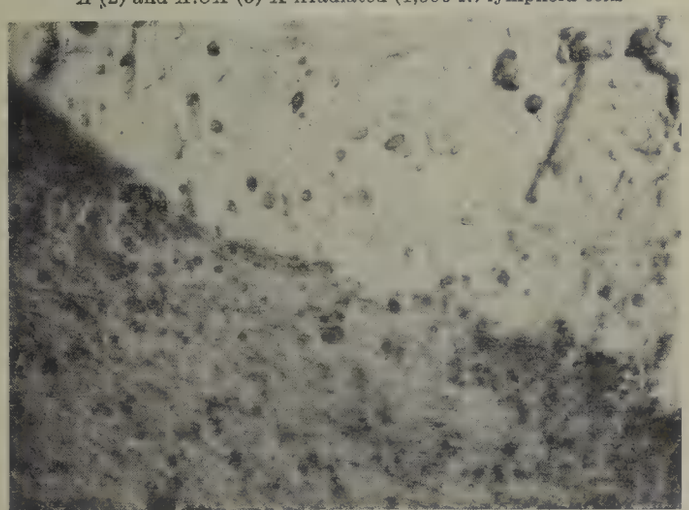

Fig. 2. Microscopic appearance of plaques on $(A \times C57BL)F_1$ mouse embryo cells subsequent to addition of irradiated *A* lymphoid cells. The destroyed monolayer is seen in the upper part of the figure, whereas the unin terrupted monolayer is seen in the lower part

cells on F_1 embryo cells: only the *A* lymphoid cells induced plaques. However, in another experiment the *C57BL* cells were efficient, again emphasizing the importance of quantitative factors in the formation of plaques.

The findings that parental lymphoid cells are capable of killing hybrid targets as a rule require a modification of the concept that *in vitro* cytotoxicity is caused by target cell contact with foreign histocompatibility antigens, since the parental lymphoid cells do not contain any foreign isoantigens. The experimental findings might be explained by two alternative possibilities: (1) Target cell death is the consequence of close contact with a structurally different but antigenetically not necessarily incompatible cell surface. Although the parental lymphoid cells do not contain isoantigens foreign to the hybrid targets, the cell surface structures are necessarily different. (2) The lymphoid cells are killed primarily by the contact with the foreign antigens on the target cells and their death affects the viability of the target cells secondarily. The correct alternative is not yet known.

Since F_1 lymphoid cells are efficient against parental embryonic target cells the possibility that plaque-formation can be ascribed to a primary immune response is unlikely. However, in the experiment with allogenic and parental lymphoid cells the necessary requirements for an immune response are present. Although the tissue culture conditions and the speed of the reaction make this possibility less likely, experiments were performed to examine the effect of heavily irradiated lymphoid cells on the monolayer cultures. The cells were given 1,500 r. which completely inhibited the phytohaem-agglutinin-induced stimulation of DNA synthesis. It was found that X-irradiation did not detectably inhibit the ability of hybrid lymphoid cells to cause plaques on parental embryo cells (Table 1, Exps. 5 and 7). Neither did X-irradiation affect the plaque-forming efficiency of allogenic and parental lymphoid cells (Table 1, Exps. 5, 7, 10). Thus, lymphoid cell division is not a necessary prerequisite for the effect.

A few experiments were performed to study the ability of erythrocytes to induce plaques on monolayer cultures. Addition of allogenic or parental erythrocytes on to embryonic targets did not result in plaques, whereas the same number of lymphoid cells were efficient in the same Petri dishes (Table 1, Exps. 1 and 4). Since red cells are known to contain considerably fewer *H*-2 isoantigenic determinants than lymphoid cells[6] it cannot be excluded that quantitative factors regarding antigenicity were responsible for the discrepancy.

The results reported substantiate the earlier conclusion that a non-immunological recognition process is initiated by the close contact between antigenically and/or structurally incompatible lymphoid cells and target neoplastic and embryonic cells, which results in damage or death

Table 1. Plaque-formation by Non-immune and Irradiated Lymphoid Cells and Erythrocytes on Monolayer Cultures of Mouse Embryo Cells

Exp. No.	Target genotype	Added cells			No. of plaques studied	Plaque formation	Latency period (days)
		Genotype	Type	Treatment			
1	*A.SW*	*A.SW*	*lgl* (1)	–	1	–	6
		C57BL	*lgl* (1)	–	1	+	6
		A.SW	Red cells	–	1	–	
		C57BL	Red cells	–	1	–	
2	*A*	*A*	*lgl*	–	1	–	
		A.SW	*lgl*	–	1	+	1·5
3	*5M*	*A*	*lgl*	–	1	+	2
		$(A \times C57BL)F_1$	*lgl*	–	1	+	2
		$(A \times C57L)F_1$	*lgl*	–	1	+	2
		DBA	*lgl*	–	1	–	
		C3H	*lgl*	–	1	–	
4	*A.SW*	*A.SW*	*lgl*	–	4	–	
		A.CA	*lgl*	–	2	+	4
		A	*lgl*	–	2	+	4
		A.SW	Spleen	–	3	–	
		A.CA	Spleen	–	2	+	4
		A	Spleen	–	2	+	4
		A.SW	Red cells	–	1	–	
		A.CA	Red cells	–	2	–	
		A	Red cells	–	1	–	
5	*A.SW*	*A.SW*	*lgl*	1,500 r.	1	–	
		A.CA	*lgl*	1,500 r	1	+	3
		$(A.SW \times A.CA)F_1$	*lgl*	1,500 r.	1	+	3
6	*C3H*	*C3H*	*lgl*	–	1	–	
		CBA	*lgl*	–	1	–	
		A	*lgl*	–	1	+	3
		5M	*lgl*	–	1	+	3
		$(A \times C3H)F_1$	*lgl*	–	1	+	3
7	*A*	*A*	*lgl*	1,500 r.	6	–	
		C57BL	*lgl*	1,500 r.	3	–	
		$(A \times A.CA)F_1$	*lgl*	1,500 r.	3	+	4
		$(A \times C57BL)F_1$	*lgl*	1,500 r.	3	–	
8	$(A \times C57BL)F_1$	$(A \times C57BL)F_1$	*lgl*	–	4	–	
		A	*lgl*	–	4	+	3
		C57BL	*lgl*	–	2	+	3
		A.SW	*lgl*	–	2	+	3
9	$(A \times C57BL)F_1$	$(A \times C57BL)F_1$	*lgl*	–	2	–	
		A	*lgl*	–	2	+	2
		A.CA	*lgl*	–	2	+	2
		$(A \times C57BL)F_1$	Spleen	–	2	–	
		A	Spleen	–	2	+	2
		A.CA	Spleen	–	2	+	2
10	$(A \times C57BL)F_1$	$(A \times C57BL)F_1$	*lgl*	1,500 r.	6	–	
		A	*lgl*	1,500 r.	4	+	5
		C57BL	*lgl*	1,500 r.	4	–	
		A.CA	*lgl*	1,500 r.	4	+	5
11	$(A \times DBA)F_1$	$(A \times DBA)F_1$	*lgl*	–	1	–	
		A	*lgl*	–	1	+	1
		DBA	*lgl*	–	1	–	
		A.SW	*lgl*	–	1	+	1
12	$(A \times CBA)F_1$	$(A \times CBA)F_1$	*lgl*	–	1	–	
		A	*lgl*	–	1	+	1·5
		CBA	*lgl*	–	1	–	
		A.SW	*lgl*	–	1	+	1·5
13	$(CBA \times A.SW)F_1$	$(CBA \times ASW)F_1$	*lgl*	–	1	–	
		CBA	*lgl*	–	1	–	
		A.SW	*lgl*	–	1	+	2
		A	*lgl*	–	1	+	2
		5M	*lgl*	–	1	+	2

lgl = lymph node cells.

of the target cells. Lymphoid cell division is not involved in the process. It appears likely that the target cells are killed as a consequence of contact with incompatible antigens or surface structures carried by the normal lymphoid cells and not by immunological reactions of conventional types. According to this concept cellular immunity might be the expression of a more general biological phenomenon, which may function as a cell-to-cell homoeostatic mechanism tending to eliminate cells with altered cell surface structures and which may be, therefore, important as a control mechanism against neoplasia and other aberrations of cell differentiation.

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MEASUREMENT OF UNSTEADY STATE GROWTH RATES OF MICRO-ORGANISMS

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THE measurement of growth rates of micro-organisms by determining the rate of change of the concentration of a cellular component such as protein, DNA, or RNA is well established[1], as is the use of dry weight or optical density measurements on the growing culture to approximate the dry weight[2]. Most frequently these techniques are applied during the phase of growth known as 'log phase', and the growth rate is determined simply by plotting the logarithm of the optical density (or dry weight, protein concentration, etc.) versus time. Because the growth rate remains constant for at least several generations under most conditions of culture, the accuracy of the estimation is increased by plotting a number of points and determining the best straight line, the slope of which is used to calculate the growth rate.

For studies of growth rates under conditions where the growth rate is changing with time, these techniques are unsatisfactory because a 'best straight line' does not exist, and estimation of the slope of a curved line is not reliable. We have found that a technique combining continuous culture with continuous indirect measurement of the cell mass concentration permits accurate estimation of unsteady-state growth rates.

The method is based on the principle of measuring continuously the concentration of a single nitrogen source in the medium on which a micro-organism is grown in continuous culture. Typically, the nitrogen source is an ammonium salt, although the use of an amino-acid or other nitrogen source would be equally satisfactory, provided that an assay exists suitable for use with a continuous analyser such as the Technicon 'AutoAnalyzer'.

By writing material balances on the growth vessel, the following equations are obtained[3]:

$$\frac{dN_c}{dt} = \mu N_c - DN_c \qquad (1)$$

and:

$$\frac{dN}{dt} = DN_0 - DN - \mu N_c \qquad (2)$$

where N represents the concentration of source nitrogen in the growth vessel, N_c represents the concentration of cellular nitrogen in the growth vessel, N_0 represents the concentration of source nitrogen in the incoming medium, μ represents the specific growth or nitrogen assimilation rate, D represents the dilution rate or the ratio of medium flow rate to medium volume contained in the growth vessel, and t represents time. In the absence of excretion of nitrogen compounds and significant changes in intracellular amino-acid pools, the nitrogen assimilation rate will correspond to the growth rate[4].

The growth rate determined in this manner would correspond to a growth rate measured by following the increase in Kjeldahl nitrogen incorporated by the cells. For the case in which the nitrogen source is made the growth-rate-limiting nutrient, the following equation[5] also applies.

$$\mu = \frac{\mu_{max} N}{K_s + N} \qquad (3)$$

in which μ_{max} represents the maximum growth rate of the cells in the presence of an excess of limiting nutrient, and K_s is a constant. For nutrient-limited conditions in a continuous culture, the concentration of limiting nutrient in the culture vessel at steady-state is independent of its concentration in the incoming medium.

When N_0 is held constant, equation (2) can be rearranged to yield the growth rate in terms of the other variables:

$$\mu = D - \frac{dN}{dt} / (N_0 - N) \qquad (4)$$

Thus, in order to calculate the instantaneous growth rate, one need only determine from the recorder-chart the value of N at some time t, estimate the value of dN/dt by numerical methods, and knowing D and N_0, calculate the growth rate, μ.

The sensitivity of this technique resides in the fact that changes in N are easier to observe and measure accurately than changes in N_c, as may be seen from Table 1.

As Table 1 shows, during the first minute after the change in growth rate, the concentration of ammonium nitrogen in the medium more than doubles while the cell nitrogen concentration falls by less than 2 per cent. The change in ammonia concentration is readily measurable with a Technicon 'AutoAnalyzer', while it would require unusually precise measurements to detect a difference in cell dry weight or optical density of 2 per cent.

We have applied this method to the problem of determining the behaviour of a microbial culture during a 'shift up'[1]. In this case the shift up was obtained by increasing the dilution rate of the culture, rather than by shifting to a richer medium capable of supporting faster growth. The organism used was *Escherichia coli B*, growing at 37° C in a well-stirred growth vessel holding 200 ml. of *M*-63 medium[6] containing glycerol as a carbon source and ammonium sulphate (63 mg of ammonium nitrogen/l.) as a nitrogen source. The medium was fed from a reservoir by a 'Sigmamotor' pump. A sample of broth and cells from the growth vessel was taken continuously by a Technicon 'AutoAnalyzer', and the concentration of dissolved ammonia was determined using an alkaline phenol-hypochlorite technique[7]. Glucose in the medium interfered with this assay and therefore glycerol was used as carbon source.

Table 1. Changes in the Concentration of Cellular Nitrogen (N_c) and Medium Nitrogen (N) in a Continuous Culture after the Micro-organism's Growth Rate suddenly falls to Zero

Time (min)	N (mg/l.)	N_c (mg/l.)
0	1	99
1	2·6	97·4
2	4·2	95·8
3	5·8	94·2

Calculations made for a continuous culture with: $D = 1{\cdot}0\ h^{-1}$; $N_0 = 100$ mg/l.

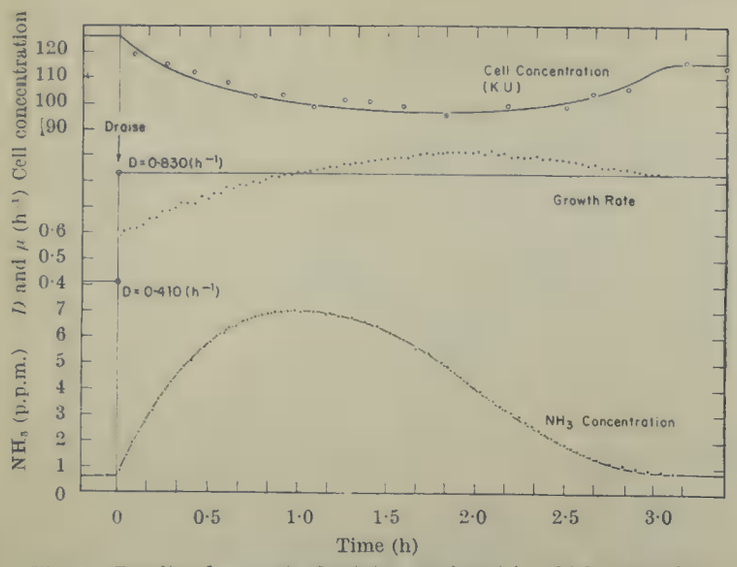

Fig. 1. Results of an unsteady-state experiment in which ammonium nitrogen was the limiting nutrient

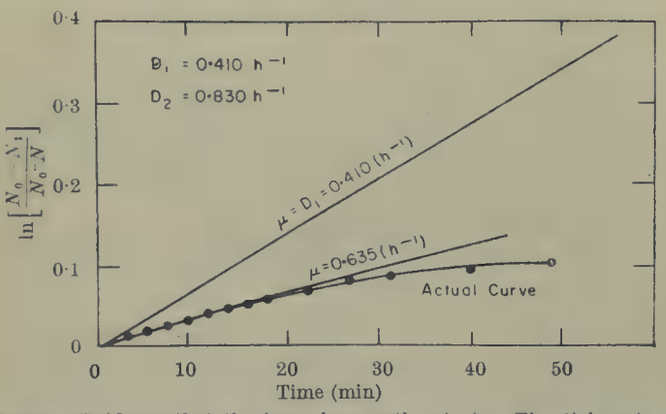

Fig. 2. Evidence that the jump in growth rate (see Fig. 1) is not an artefact. N_1 represents the steady-state concentration of ammonium nitrogen in the growth vessel prior to the increase in dilution rate

Prior to the unsteady-state growth rate measurements, a steady state was maintained for at least 15 h. Then an increase in dilution rate was made, the concentration of ammonium nitrogen determined as a function of time after the step change in dilution rate, and the instantaneous growth rate calculated from data obtained from the plot of N versus time. The calculations were carried out on an IBM 7094 digital computer because they involved the manipulation of considerable data.

The results of a typical run are shown in Fig. 1, for which $D_1 = 0{\cdot}41$ h^{-1} and $D_2 = 0{\cdot}83$ h^{-1}. It appears that the growth rate rose about 0·2 h^{-1} immediately after the dilution rate change and then slowly rose further, overshooting a new steady-state growth rate, and then settling back into a steady state. That the instantaneous rise is real, and not an artefact introduced through numerical differentiation, is shown in Fig. 2, in which $\ln(N_0 - N_1/N_0 - N)$ versus time is plotted. The slope of the curve is equal to $D - \mu$ (ref. 8). The solid line indicates the results that would be obtained if the growth rate remained at the value it had prior to the dilution rate change. Since the actual line is not tangent to the solid line, it indicates that a real increase in growth rate occurred at the time of the dilution rate increase, and since the line has a constant slope for about 15 min, that the growth rate stayed constant at 0·63 h^{-1} for about 15 min before increasing further over the course of the next few hours.

Table 2 shows the results of a series of experiments made at a number of dilution rates and dilution rate changes. For small dilution rate changes, the perturbation in ammonium nitrogen concentration was absent or small, indicating that the growth rate accommodated the dilution rate change, whereas when the dilution rate change increased, the ability of the cells to increase their growth rate was not sufficient and a large perturbation in ammonium nitrogen was observed.

Table 2. Results of Step Response on Nitrogen-limited Culture

	1	2	3	4	5	6	7
ΔD (h^{-1})	0·18	0·19	0·19	0·29	0·42	0·47	0·48
D_1 (h^{-1})	0·286	0·316	0·525	0·492	0·410	0·377	0·388
D_2 (h^{-1})	0·466	0·503	0·710	0·777	0·830	0·845	0·870
Maximum deflexion of N (mg/l.)	–	–	–	1·7	7·0	11·5	23·2
Time at which N reaches max (min)	–	–	–	35	60	70	130
Time to reach new steady state (h)	–	–	–	1·25	3·0	4·6	12·5
μ_{max} (h^{-1})	–	–		1·0	0·91	0·93	1·0
Comment	No deflexion		Very small deflexion				

In order to determine whether Monod's equation (equation (3)) could be used to represent unsteady-state growth rates, equations (1), (2) and (3) were solved by a numerical method using the digital computer. Regardless of the magnitudes of the constants μ_{max} and K_s or the parameters N_0 and D, the transient growth rate calculated on the assumption that Monod's equation is applicable always lacks a maximum and is a smooth transition between the two steady states[9]. Clearly the experimental curve (Fig. 2) departs significantly from Monod's model. Thus, during transient operation of a continuous culture, Monod's equation may not be used to relate growth rates and concentrations of limiting nutrient.

In contrast to the results of Kjeldgaard, Maaløe and Schaechter[1], we have found that the cell can increase its rate of protein synthesis immediately after a shift up, and that this small increase is then followed by a prolonged gradual increase. The discrepancy between the present findings and the earlier ones may be ascribed to the relative insensitivity of the direct measurement of the growth rate by following changes in optical density, or protein concentration as practised by the earlier workers.

The possibility that the immediate increase in the nitrogen assimilation rate, here interpreted as the growth

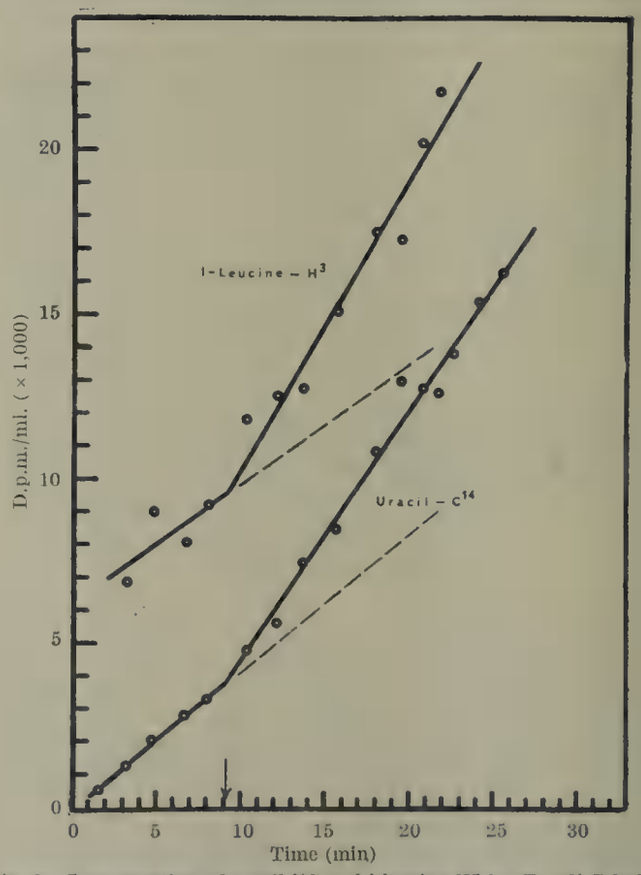

Fig. 3. Incorporation of uracil-^{14}C and l-leucine-^{3}H by *E. coli B* before and after change in dilution rate. The medium contained 10 mg of l-leucine and 10 mg of uracil per litre in addition to the ingredients contained in *M*-63 medium used in the previous experiment. Ten μc. of l-leucine-^{3}H and 10 μc. of uracil-^{14}C were added to the culture 9·1 min prior to the change in dilution rate. At the time indicated by the arrow, the dilution rate was increased from 0·45 h^{-1} to 1·16 h^{-1}. Samples were taken approximately at 1-min intervals, added to an equal volume of ice-cold 5 per cent TCA, and at the conclusion of the experiment filtered through a 'Millipore' filter, washed, and counted by a liquid scintillation counter. The counts were corrected for dilution of the radioactive tracer in the culture vessels by incoming medium containing only unlabelled l-leucine and uracil, and for the cell concentration

rate, represented a preferential increase in the rate of RNA synthesis alone rather than an increase in the rate of both protein and RNA synthesis can be excluded on the basis of the experimental results depicted in Fig. 3. The results clearly indicate that at the time of the change in dilution rate, the rate of incorporation of both 1-leucine-^{3}H and uracil-^{14}C increased sharply, implying that the rates of protein and RNA synthesis increased.

The results recorded here further indicate that protein and RNA biosynthetic activities in the cell are not at the maximum levels of their capacities under the given conditions, since the rate of nitrogen assimilation is increased immediately when the feed rate of limiting nutrient is increased.

In the experiments described here, it was found that all the nitrogen in the medium could be accounted for as free ammonium nitrogen; the cells did not excrete any intermediary products of nitrogen metabolism. This might not always be true, particularly under conditions in which the carbon source is the limiting nutrient, and it would be desirable to make certain that the nitrogen in the medium is all in the form which is being assayed. This can be done by collecting on ice a portion of effluent, centrifuging to remove cells, and comparing the ammonium nitrogen analysis of the cell-free medium with a total nitrogen analysis.

These results clearly indicate that, contrary to the views of Herbert[10], a significant lag in adjustment of growth rate to dilution rate does exist, and that this lag must be taken into account in experimenting with single or multistage continuous culture systems. For example, determining the apparent maximum growth rate (μ_{max}) from the slope of a plot of ln x versus t may underestimate the true μ_{max}.

It should be noted that it is not necessary for the nitrogen source to be the limiting nutrient in order to use this technique for studying unsteady-state growth rates. All that is required is for the concentration of the nitrogen source in the growth vessel to be low relative to its concentration in the reservoir. This could be accomplished, for example, by having the carbon source as limiting nutrient and using a concentration of nitrogen source in the medium sufficient to leave five or ten parts per million unused under steady-state condition. For most cases, this concentration will be in excess, that is, the growth rate of the organism will not be affected by small changes in the concentration of nitrogen source. Using this technique it would be possible to study the effects on growth rates of concentrations of inhibitory substances too low to study by standard techniques, because they would not stop growth entirely but only reduce the growth rate slightly for short periods of time.

The calculations were performed on an IBM 7094 digital computer at the Computation Center of this Institute.

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PROTONATION EQUILIBRIA AND ALKALINE HYDROLYSIS OF GLYCINE ETHYL ESTER

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IN the past decade, interest has developed in the interaction of metal ions with biologically important compounds, for example, amino-acid esters and peptides. The complexing tendencies of such molecules, the stabilities and reactivities of the resulting complexes have been investigated[1–3], but very little systematic work has been carried out. The following account describes preliminary work towards such an investigation.

The proton ionization equilibria of glycine ethyl ester and glycine, and the alkaline hydrolysis of the ester, have been examined prior to a detailed analysis of the metal ion complexing and catalysis.

The ionization constants were measured at 25° ± 0·05° C and 0° ± 0·05° C; and at three differing ionic strengths in the case of the ester. Values for the pK_a of glycine[4] have been reported and temperature coefficient data[5] are available. The glycine work was therefore carried out mainly as a check on the accuracy of the ester measurements. *p*H titrations were carried out using a Radiometer *p*H-stat titrator, coupled with a Radiometer *p*H scale expander. Alkali was added from an all-glass micrometer syringe. The *p*H assembly was operated manually. Where necessary, the ionic strength was brought to the required value by addition of potassium bromide solution. pK_{a_1} at 25° C for glycine was estimated by decreasing the *p*H of the initial solution to approximately 2·5 by addition of hydrochloric acid. pK_a values were calculated from selected points (on the average, sixteen points were chosen) along the titration curve using conventional methods[6]. An average of five titrations were performed for each ionic strength. *p*H values were converted to concentrations using the Davies equation[7], and equilibrium constants thus calculated (Table 1) are concentration quotients. For the ester, drifting values, which were a result of hydrolysis, were obtained at high *p*H.

pK_a values independent of ionic strength are expected in the ionization of glycine ethyl ester ($H^+E \rightarrow H^+ + E$), and this is confirmed by the results. The scatter in values at 25° C is much higher than at 0° C. This is expected as a consequence of the increased rate of hydrolysis at 25° C. The average value at 25° C, 7·72, agrees well with 7·73 reported by Emerson and Kirk[8], and with an estimated value of 7·76 (ref. 9) based on a ΔpK ($\dot{N}H_3CH_2COO^- -$

Table 1

Acid	Temp.	Ionic strength	pK_{a_1}	pK_{a_2}
Glycine ethyl ester hydrochloride	25° C	0·05	$7·71_7 \pm 0·01$	—
		0·075	$7·74_4 \pm 0·01_5$	—
		0·100	$7·70_0 \pm 0·02_3$	—
	0° C	0·01	$8·35_6 \pm 0·00_8$	—
		0·10	$8·40_8 \pm 0·01_9$	—
Glycine	25° C	0·1	$2·33_6 \pm 0·02$	$9·61_5 \pm 0·01_4$
	0° C	0·1	—	$10·30_5 \pm 0·00_6$

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$\overset{+}{N}H_3CH_2COOEt$) of 2·02. It is also close to the value for the methyl ester, 7·66, found by Edsall[10].

pK_a values strongly dependent on ionic strength are expected for the second ionization of glycine ($H^{\pm}G \rightleftharpoons H^+ + G^-$), and approximately independent of ionic strength for the first ionization ($H^+G \rightleftharpoons H^{\pm}G + H^+$). Thus for comparative purposes a thermodynamic pK_{a_2}, $9{\cdot}84_8$, was calculated from the second ionization using the equation:

$$\log K_{T\cdot D} = \log K_c - \frac{2Az^2\sqrt{I}}{1+\sqrt{I}} + 0{\cdot}2z^2I$$

This value compares favourably with that of $9{\cdot}78_0$ (ref. 5), as does the value reported here, $2{\cdot}33_6$, with that listed[5], $2{\cdot}35_0$, for the first ionization.

Table 2 lists $\Delta G^{\ominus}$, $\Delta H^{\ominus}$, $\Delta S^{\ominus}$ and $T\Delta S^{\ominus}$ values for the ionizations, assuming $\Delta H^{\ominus}$ independent of T from 0° C to 25° C, along with analogous values calculated from Edsall's[10] data on glycine methyl ester over the smaller temperature range (20°–30° C), and from tabulated data[5] for the second ionization of glycine.

Differences in pK_a for the processes

$$\overset{+}{N}H_3CH_2COOEt(aq) \rightleftharpoons NH_2CH_2COOEt(aq) + H^+(aq)$$

$$\overset{+}{N}H_3CH_2COO^-(aq) \rightleftharpoons NH_2CH_2COO^-(aq) + H^+(aq)$$

are predominantly due to a difference in $\Delta S^{\ominus}$ for the ionization process. Qualitatively a process, electrically neutral species → 2 ions, would be expected to have a more negative $\Delta S^{\ominus}$ than the process ion → ion. This is, in fact, observed, while no great difference in $\Delta H^{\ominus}$ is found. Values of $\Delta S^{\ominus}$ for the ionization of carboxylic acids[11] (for example, −22 cal mole^{-1} deg.$^{-1}$ for acetic acid) are considerably more negative than that for the second ionization of glycine. The low value of $|\Delta S|$ for glycine seems related to the existence of the dipolar zwitterion. Comparable $\Delta S^{\ominus}$ values to that of the ester are found in monobasic amine acids[11].

The alkaline hydrolysis of glycine ethyl ester has been investigated at an ionic strength of 0·1, and at 25° C and 0° C over a wide pH range. The kinetics were followed by a pH-stat device which enables the pH to remain constant by automatic and recorded addition of alkali. The accuracy of the results is limited by the accuracy with which the machine can maintain a constant pH. The following equilibria and reactions are involved:

$$\overset{+}{N}H_3CH_2COOEt(aq) \overset{K_1}{\rightleftharpoons} NH_2CH_2COOEt(aq) + H^+(aq) \quad (1)$$

$$\overset{+}{N}H_3CH_2COO^-(aq) \overset{K_2}{\rightleftharpoons} NH_2CH_2COO^-(aq) + H^+(aq) \quad (2)$$

$$\overset{+}{N}H_3CH_2COOEt(aq) + OH^-(aq) \overset{k^*}{\rightarrow} \overset{+}{N}H_3CH_2COO^-(aq) + EtOH(aq) \quad (3)$$

$$NH_2CH_2COOEt(aq) + OH^-(aq) \overset{k}{\rightarrow} NH_2CH_2COO^-(aq) + EtOH(aq) \quad (4)$$

It follows that the total alkali uptake, assuming the reaction to go to completion, is given by the expression:

$$V_\infty = [\text{ester}]_{\text{initially}} \left(1 + \frac{1}{1+\frac{K_1}{[H^+]}} - \frac{1}{1+\frac{K_2}{[H^+]}}\right)$$

All pseudo first-order rate plots were calculated on this basis. At high pH, where the runs were taken to completion, good agreement with the observed V_∞ was obtained. Second-order rate constants were estimated from the pseudo first-order rate constants by division by the hydroxide ion concentration calculated from the pH of the run (or average pH if drifting with time occurred) and the Davies equation[7]. Second-order rate constants were found to vary with hydrogen ion concentration. The pH range extended from 9·6 to 11·7. Fig. 1 gives the results for some 50 runs at 25° C.

Table 2

	Glycine ethyl ester hydrochloride		Glycine second ionization	
$\Delta G^{\ominus}_{298}$ kcal mole^{-1}	+10·45	+10·50*	+13·06	+13·16†
$\Delta H^{\ominus}$ kcal mole^{-1}	+9·7	+12·10*	+10·6	+11·36†
$T\Delta S^{\ominus}$ cal mole^{-1} °K^{-1}	−2·7	+5·6*	−8·6	−6·1†
$T\Delta S^{\ominus}_{298}$ kcal mole^{-1}	−0·8	+1·7*	−2·5	−1·8†

* Glycine methyl ester—Edsall (ref. 10).
† Robinson and Stokes: *Electrolyte Solutions*.
$\Delta G^{\ominus}$ calculated from $-RT\ln K$.

Table 3

Temp.	Protonated ester	Non-protonated ester
0° C	$k^* = 230$	$k = 8{\cdot}5$
25° C	$k^* = 1{,}130$	$k = 35{\cdot}6$
	$E_A^* = 10{\cdot}6$	$E_A = 9{\cdot}4$
	$A^* = 7{\cdot}4 \times 10^{10}$	$A = 3 \times 10^8$
	$\Delta S^{*\ddagger}_{298} = -12$	$\Delta S^{\ddagger}_{298} = -23$

Rate constant in units: 1 mole^{-1} min^{-1}.
Activation energy in units: kcal mole^{-1}.
Entropy of activation: cal mole^{-1} ° K^{-1}.

If the kinetics follow the scheme outlined previously and corrections have been made for the protonation equilibria involved:

$$k_{\text{obs}}\, a = k^*[H^+E] + k[E]$$

where a represents the initial total ester concentration (that is, $[H^+E]_0 + [E]_0$). If, to a first approximation, $[E] = a$, then:

$$k_{\text{obs}} = k + \frac{(k^* - k)\,[H^+]}{K_1}$$

A higher approximation would give:

$$k_{\text{obs}} = k + (k^* - k)\frac{[H^+]}{K_1}\left\{1 - \frac{[H^+]}{K_1}\right\}$$

Such a correction is well within the experimental errors and, therefore, pointless.

Thus an observed rate constant varying with $[H^+]$ should be found, and a plot of k_{obs} V $[H^+]$ should give k as intercept, and k^* calculable from the gradient. Table 3 summarizes the data for experiments at 25° C and for a few experiments at 0° C. Provisional estimates of E_A, A, $\Delta S^{\dagger}$ are given.

The Bjerrum[12] type equation representing the effects of electrostatic interactions between a charged substituent and the reaction site in the transition state is:

$$\frac{k^*}{k} = \exp\left(\frac{-z_iz_je^2}{D\mathbf{k}TR}\right) \quad (5)$$

where k^* and k are the rate constants for reactions involving the charged molecule and uncharged molecule, respectively, z_ie is the charge on the initial molecule, and z_je is the charge developed at the reaction site. R is the distance between the charges.

Thus:

$$E^* - E = \frac{Ne^2z_iz_j}{DR}\left[1 + \frac{T}{D}\frac{dD}{dT}\right] \quad (6)$$

where E^* and E are the activation energies for reactions involving charged and uncharged molecules, respectively. N is Avogadro's number.

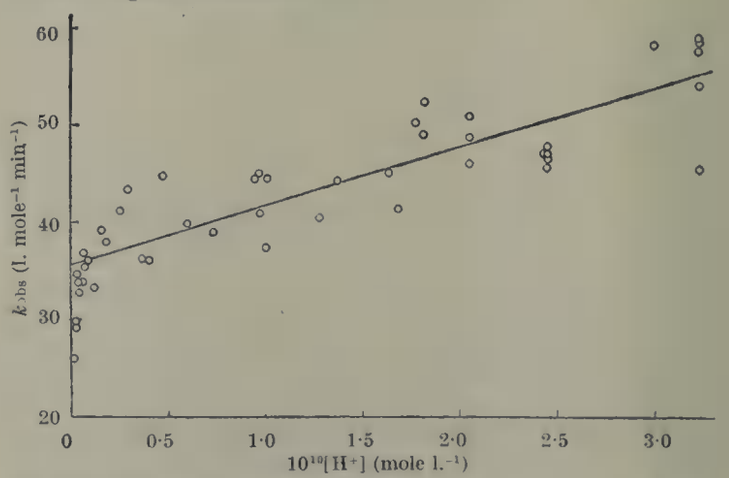

Fig. 1. Variation of observed rate constant with hydrogen ion concentration at 25° C

As:

$$\left(1 + \frac{T}{D}\frac{dD}{dT}\right) < 0$$

for aqueous solutions, $E^* >$ or $< E$ depending on whether z_i and z_j are of unlike or like sign, respectively. For the system reported here $z_i z_j < 0$, and thus $E^* > E$. The preliminary results indicate that this may be so. This effect has been noted previously for $z_i z_j < 0$ (ref. 13) and $z_i z_j > 0$ (ref. 14). The present case, however, provides a much more direct test of effects of charges in so far as the two molecules being compared ($\overset{+}{N}H_3CH_2COOEt$, NH_2CH_2COOEt) differ, apart from charge, only in respect of the hydrogen. With substances investigated previously the comparison made was of the type $SO_3^-(CH_2)_nCOOEt$ with $CH_3(CH_2)_nCOOEt$.

From equation† (5) a value of R, 1·8 Å, is found assuming D to be the macroscopic dielectric constant. This can, in turn, be used to find $E^* - E$ equal to approximately 1·0 kcal mole^{-1}. Further refinements using a Kirkwood–Westheimer[15]–Tanford[9] procedure indicate a value of approximately 1·6 kcal mole^{-1} ($D_E = 14$, $R = 4{\cdot}85$ Å).

The effect of charge on rate should be apparent in the pre-exponential terms. For reaction involving the charged species, formation of the transition state involves a decrease in net charge and thence an increase in entropy, due to release of water molecules of solvation on formation of the transition state. A less negative $\Delta S^{\ominus}$, relative to that for the uncharged species, is expected. This is tantamount to saying that there is an enhanced probability of reaction (relative to a neutral molecule–ion reaction) when two ions of opposite sign approach each other.

The alkaline hydrolysis of glycine esters has been examined previously, but no variation with pH has been noted. Bell and Coller[16] investigated the hydrolysis over the pH range 9·70–11·55, but found no pH dependence. Other authors did not carry out the reaction at varying pH values. Reported values of the rate constant for the hydrolysis at 25° C are: (*a*) Bell and Coller[16], 50·4 l.mole^{-1} min^{-1}; (*b*) Gustafsson[17], 36 l.mole^{-1} min^{-1}; (*c*) White, Manning and Li[2], 44 l.mole^{-1} min^{-1}; (*d*) Gelles and Robson[18] (for methyl ester), 39 l.mole^{-1} min^{-1}.

Conditions for values (*b*) and (*c*) are not reported, but from the experimental technique it seems safe to conclude that the pH was probably greater than 10. These data are thus consistent with the present investigation and agree well with work[18] on the methyl ester.

It can be seen that the effect of temperature on the overall observed rate, and on the gradient of the k_{obs} V $[H^+]$, is complex; depending on the temperature coefficients of k, k^* and K_1. Knowing these coefficients it is possible to predict that the pH dependence will be larger (and consequently more easy to measure) at temperatures > 25° C, and work is in progress to extend the measurements.

Preliminary work has been carried out on the cupric ion catalysis. Titration curves for the ester in the presence of varying quantities of cupric ion suggest the presence of complexes such as CuE, CuE_2.

Preliminary kinetic work shows that in addition to the hydroxide ion catalysed hydrolysis, a series of consecutive pseudo first-order reactions are occurring which can be initially interpreted as:

$$E + Cu^{2+} + OH^- \xrightarrow{k_1}$$
$$E + CuG^+ + OH^- \xrightarrow{k_2}$$
$$E + X + OH^- \xrightarrow{k_3}$$

where CuG^+ is copper monoglycinate, and X is some, as yet, undetermined reactant. This is analogous to previous work[18] where a maximum in the rate was obtained at pH 7–8 for k_1, k_2 and k_3.

I thank Dr. E. Gelles for suggesting this topic, and Dr. P. G. Wright for helpful discussion. This work was carried out during the tenure of the Edward A. Deeds junior fellowship of Queen's College, Dundee.

† Values of the rate constants at infinite dilution were calculated, prior to substitution into equation (5).

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DIFFUSIVE TRANSPORT RATES IN STRUCTURED MEDIA

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THE purpose of this short article is to call attention to the importance and potential applications of the abnormal diffusive transport rates recently observed in suspensions and in polymeric solutions and to offer a tentative interpretation of these phenomena.

The experimental results obtained for a variety of systems are given in Table 1; it is not the purpose of this article to present a complete review of all available data and additional results are given both in the references quoted in Table 1 and in other related papers[1,2]. The important point to be made is that not infrequently the addition of either suspended solids or polymeric material to a homogeneous liquid increases the diffusive transport rates of some third species in this medium. This is not the case for all systems or at all concentration-levels, but when it does occur the changes may be appreciable (up to 40 per cent in the case of the data of Table 1, and significantly larger effects have been noted[3]). Presumably, even greater effects would be possible by optimizing the relevant factors involved, if these were understood, since the data given in Table 1 represent only measurements on systems chosen more or less at random rather than with the view of finding maximal effects of this kind. As the diffusivity increases are obtainable using either suspended solids or dissolved macromolecules, the total effects obtainable in biological media containing both kinds of constituents could conceivably be very appreciable and significantly affect the rates of respiratory and cellular ingestive processes of living organisms or the operability of machines designed to provide such functions. Thus the problem under discussion may be of interest in rather broad areas of biophysics and bioengineering as well as in polymer and colloid science.

Students of the diffusive behaviour of polymeric molecules in solution have observed or predicted effects which may have a common molecular origin, although, except

Table 1. Measured Diffusivities

Diffusing solute	Medium used	Temp. (° C)	Diffusivity, (cm^2/sec $\times 10^5$)	Investigators
Ethylene	Water	18	~1·10	Astarita[15,16]
Ethylene	0·5% CMC*	18	1·06	Astarita
Ethylene	1·0% CMC	18	1·08	Astarita
Ethylene	2·0% CMC	18	1·29	Astarita
Carbon dioxide	Water	18	1·72	Astarita
Carbon dioxide	0·122% Carbopol†	18	1·82	Astarita
Carbon dioxide	0·132% Carbopol	18	1·82	Astarita
Carbon dioxide	0·172% Carbopol	18	1·98	Astarita
Carbon dioxide	0·222% Carbopol	18	2·16	Astarita
Carbon dioxide	0·375% CMC	18	1·72	Astarita
Carbon dioxide	0·75% CMC	18	2·06	Astarita
Carbon dioxide	1·50% CMC	18	2·44	Astarita
Carbon dioxide	3·80% Bentonitic clay in water	18	1·91	Astarita
Carbon dioxide	5·01% Bentonitic clay in water	18	2·02	Astarita
Carbon dioxide	5·10% Bentonitic clay in water	18	2·40	Astarita
Benzoic acid	*Cyclo*hexane	25	1·18	Hopper[13]
Benzoic acid	2% PIB‡	25	1·35	Hopper
Benzoic acid	4% PIB	25	0·92	Hopper
Acetic acid	Water	20	1·24	Perry[17]
Acetic acid	4% CMC	20	1·63	Heertjes *et al.*[18]

* CMC denotes carboxymethylcellulose (wt. per cent given) dissolved in water.

† A water soluble synthetic polymer; solution concentrations studied as indicated.

‡ Two wt. per cent of polyisobutylene dissolved in *cyclo*hexane.

in the case of a small number of lucid publications[4–6], the actual phenomenon has frequently been obscured by the dedicated devotion of the authors in this area of science to the reporting of phenomena in terms of empirical coefficients of obscure origin and value, as well as by the fact that the macromolecular transport rates are, at best, frequently quite low. The transport characteristics of systems in which the diffusivity increases with increasing concentration of the polymeric molecules involved have been shown to be closely related to the second virial coefficient of the solution and hence, among other variables, to the partial molal volume of the polymeric solute[4–7]. However, the extension of these considerations to make it possible to predict the behaviour of an ordinary (that is, not macromolecular) solute, in a polymer solution or a colloidal suspension, would appear to require additional considerations.

Such considerations appear to be embodied in a recent analysis of diffusion in liquids[8] which enables the prediction of diffusivity in terms of the molecular geometry (size) of both the solute and the solvent constituents and the intermolecular force fields of each. If, as indicated by the high values of the second virial coefficient of the polymeric solutions of interest, the molecular 'packing' of the solvent molecules in the neighbourhood of a macromolecule is lower than in the bulk of the solution, a region of low resistance to mass transport will have been provided. In fact, since the term reflecting such decreased intermolecular packing appears in an exponential[8], small changes in intermolecular spacing would be predicted to have major effects on the diffusivity. Similarly, in the case of diffusion in a slurry of colloidal particles, the intermolecular packing at the particle surface would be expected to be less than in the bulk of the fluid just from the usual geometric considerations of molecular packing at all planar surfaces. The visualization of the transport process therefore becomes one in which the addition of certain polymeric materials or colloidal solids to a continuous fluid may lead to the creation of a structured medium in which the packing of the fluid molecules in the immediate neighbourhood of the added macromolecule or particle is lower than in the continuum itself; these regions of low packing may represent regions of very high 'conductivity' for the diffusing species, depending in turn on their size and on the intermolecular force fields of the several species involved. The macromolecular or colloidal particles, of course, may also block the path of a diffusing species when the low-density 'short circuit' around the particles does not exist, as discussed elsewhere[1], and either mechanism could predominate, depending on the molecular force fields involved. The magnitude of the diffusivity in a 'high conductivity' environment of the added species may in principle be predicted by extensions of the previous analysis of diffusion in liquids, although in practice several difficult steps remain to be overcome before the extension is a quantitative one.

The effect of regions of abnormally high or low diffusivity on the macroscopic diffusivity of the medium as a whole may be computed from the analogous analysis of conduction of electricity or heat in structured media[9–12], providing the distribution of these regions is random and the particle asymmetry may be considered adequately. A random distribution of these regions would appear to be reasonable in an undeformed fluid, but imposition of rapid rates of deformation of the fluid could be expected to lead to asymmetric mass transport properties. This problem has been investigated in steady laminar shearing flow fields over rather broad ranges of shear rate, using both suspensions and polymeric solutions, and no measurable effects have been found[3,13]. The implications of this apparent inconsistency are not known at present.

Finally, it should be noted that the conductivity of the medium may become independent of that of the high conductivity phases or regions if the ratio of conductances is sufficiently great[11] and, if this is presumed to be the case, some predictions of the diffusivity characteristics of the structured medium may be made without detailed knowledge of the actual diffusivity in the regions in the immediate neighbourhood of the dispersed matter.

In summary, it is seen that diffusive transport rates in liquids may be increased appreciably by creation of structured media through the solution of macromolecular species in the fluid or by the suspension of colloidal particles in it. Presumably, even much greater effects than those noted so far could be obtained by optimizing the relevant parameters. These effects would appear to have potentially important implications in those areas of physics and engineering involving biological or polymeric materials. A tentative hypothesis, which assumes the creation of a structured fluid medium having regions of high diffusivity in the immediate vicinity of the added particles or molecules, is advanced as a possible explanation of this phenomenon. Methods of developing this hypothesis, which proposes a mechanism analogous to that of conduction of heat or of electricity in disperse systems or of diffusion in crystalline solids[14], are discussed.

This work has been supported by the National Science Foundation. I thank Prof. Astarita and Dr. Secor for letting me see their papers prior to publication, and Prof. Astarita for many stimulating discussions of the problem.

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CONCENTRATION EFFECTS IN CHROMATOGRAPHIC ANALYSIS

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DURING an investigation of the highly complex reaction of ω-chloroacetylamino dyes with wool, thin-layer and paper chromatography have been widely used for the detection and identification of the reaction products. From time to time it was noticed that with the dyes used the R_F values, relative to one another, varied considerably, although the final order of separation with a given mixture using a particular solvent was the same. R_F values were observed to be dependent on the concentration of material initially loaded on to the paper. A number of workers have referred to such concentration effects. Åkerfeldt[1] found that the final area of the spot was directly proportional to the load of material applied. Fowler[2], using sucrose, found a linear relationship between the logarithm of the spot length and the logarithm of the load initially applied. He also observed that at low concentrations the plot of spot length against the logarithm of the load applied was linear. Fisher, Parsons and Morrison[3], discussing quantitative paper chromatography, also found a linear relationship between spot length, or spot area, and the logarithm of the spot content when using certain amino-acids and the sugars xylose and arabinose. Miyaki[4] found that the logarithm of the difference between the initial and final lengths of the spot was a linear function of the concentration of material initially present in the spot. The effect we have observed is, however, not so much an increase in the length of the spot but a definite increase in R_F with increase in the load applied.

Giddings and Keller[5] developed an equation relating a given number of molecules Nx in a spot, the distance moved, and the mean diffusion coefficient of the particular material in the support. We have sought to explain in rather different terms the concentration effects we have observed using an equation based on present-day theories of the adsorption of dyes by textile substrates.

It is considered that the effects arise from a Freundlich type of adsorption of the dyes by the support medium. The literature contains references to the desirability of avoiding the use of supports towards which the compounds under examination show strong substantivity, but no account seems to have been taken of the possibility of changes in the pattern of chromatographic behaviour arising from relatively weak substantivity effects.

If it is assumed that the distribution of substance between the eluent and the support obeys a Freundlich isotherm, and if D_F denotes the concentration of adsorbed material in equilibrium with D_S, the concentration of the substance in the eluant, then:

$$D_F = K_1 D_S^x$$

where K_1 is a constant and $x < 1$.

If it is further assumed that the velocity with which the substance under examination moves relative to the solvent front is proportional to the concentration not adsorbed, then:

$$R_F = K_2 D_S$$

Since the total concentration of the substance in the spot $\bar{D}$ is equal to $D_F + D_S$, then:

$$\bar{D} - D_S = K_1 D_S^x$$

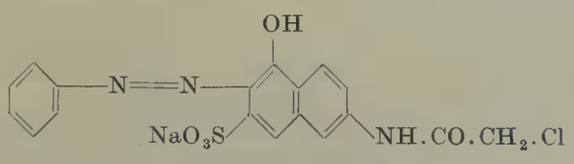
$$= \frac{K_1}{K_2^x} R_F^x$$

and if $\bar{D} \gg D_S$, that is, quite a high degree of adsorption occurs, then:

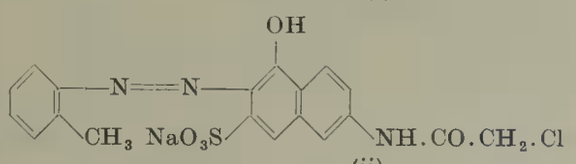
$$\log \bar{D} = \log \frac{K_1}{K_2^x} + x \log R_F$$

Thus a linear relationship should exist between the logarithm of the R_F value and the logarithm of the load, if the substantivity for the support medium of the substance loaded is sufficiently high. The accuracy of this prediction will vary according to the scale of the error introduced by assuming $\bar{D} \gg D_S$. With the low solvent/support ratios obtained in chromatography this should, in fact, even hold with compounds which show only moderate substantivity to the particular support medium. Certainly using azo dyes of the type described here and paper as the support, $\bar{D}$ will in most circumstances exceed D_S quite considerably.

Two orange dyes were used:



(i) Mol. wt. 442



(ii) Mol. wt. 456

Chromatograms were run using 'Whatman 3*MM*' paper as support, *n*-butyl alcohol/acetic acid/water (4 : 2 : 1) as the eluant and enclosing the chromatogram in an all-glass Shandon tank, containing, in the bottom, sufficient eluent to saturate the atmosphere in the tank. All the runs were continued for 21 h at 20° C. For each dye spots of equal area were formed on the starting line. The distance between the starting point and the maximum concentration point of each spot was measured. An EEL densitometer was used to determine the maximum concentration points. Since the behaviour of both dyes at each concentration was determined at the same time under identical conditions, the distances moved were equivalent to the R_F values. The results are shown in Table 1.

Fig. 1 shows the plot of the logarithm of the distance against the logarithm of the load. In both cases the predicted linear relation was obtained up to an initial load of about 0·1200 mg.

Further evidence that substantivity plays a major part in determining the movement of the spots was obtained by examining the effect of different eluents. Using *n*-butyl alcohol/pyridine/water (1 : 1 : 1), under which conditions

Table 1

Concentration of original dye solution (mg) $\bar{D}$	Distance (L) moved (cm) Dye (i)	Dye (ii)
0·0125	16·80	16·10
0·0250	17·50	16·75
0·0375	18·20	17·20
0·0500	18·50	17·50
0·0625	19·40	17·80
0·0750	19·10	18·00
0·0875	19·60	18·20
0·1000	19·10	18·40
0·1125	19·80	18·40
0·1250	19·80	18·50
0·1370	19·75	18·50
0·1620	20·00	18·50
0·1750	20·10	18·60
0·1870	20·00	18·60

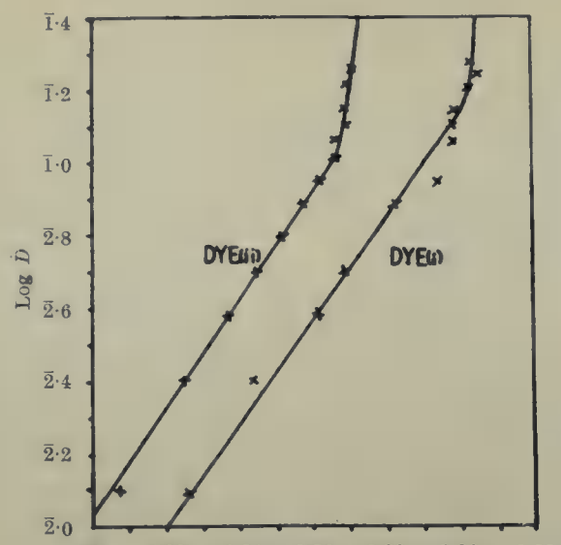
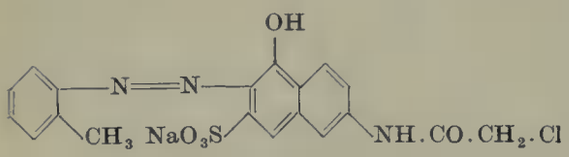


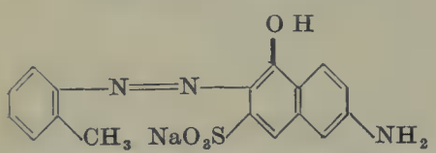
Fig. 1

hydrogen bonding of the dye to the substrate, and hence dye-substrate sensitivity, is reduced, the spots were far more compact, but the distances travelled were still dependent on concentration, though to a lesser extent.

The concentration of dye in the load has also been found to influence the chromatographic behaviour of mixtures of dyes:

Descending chromatograms were run for 21 h in *n*-butyl alcohol/acetic acid/water (4 : 1 : 2) under the same conditions as before. The dyes used in these experiments were:


(R.D.)


(H.D.)

Spots of equal area containing these dyes in different proportions were produced as follows:

[R.D.] constant (1)
[H.D.] increasing (from right to left)

The resulting chromatogram is shown in Fig. 2.

It will be seen that the expected increased movement of dye H.D. with increasing concentration occurred. Dye R.D. behaved in exactly the same way even though it was applied in constant concentration. (Note: dye H.D. is the spot with the lower R_F value.)

[R.D.] increasing (right to left) (2)
[H.D.] constant

The resulting chromatogram is shown in Fig. 3. In this case no obvious mixture effects were observed except that a slight retarding of the movement of dye R.D. is observed when this result is compared with that shown in Fig. 4 (*a*).

[R.D.] increasing (left to right) (3)
[H.D.] increasing (right to left)

The resulting chromatogram is shown in Fig. 5. The position of the spots (on the extreme left) containing most dye H.D. and least dye R.D. shows that dye R.D. is held back. Particularly marked separation of the spot containing most dye R.D. and least dye H.D. occurred (on extreme right of Fig. 5).

It is obvious that the chromatograph behaviour of mixtures of dyes is affected by differences in the substantivity of each dye for the support. That this is the case with the adsorption of direct dyes on cellulose has

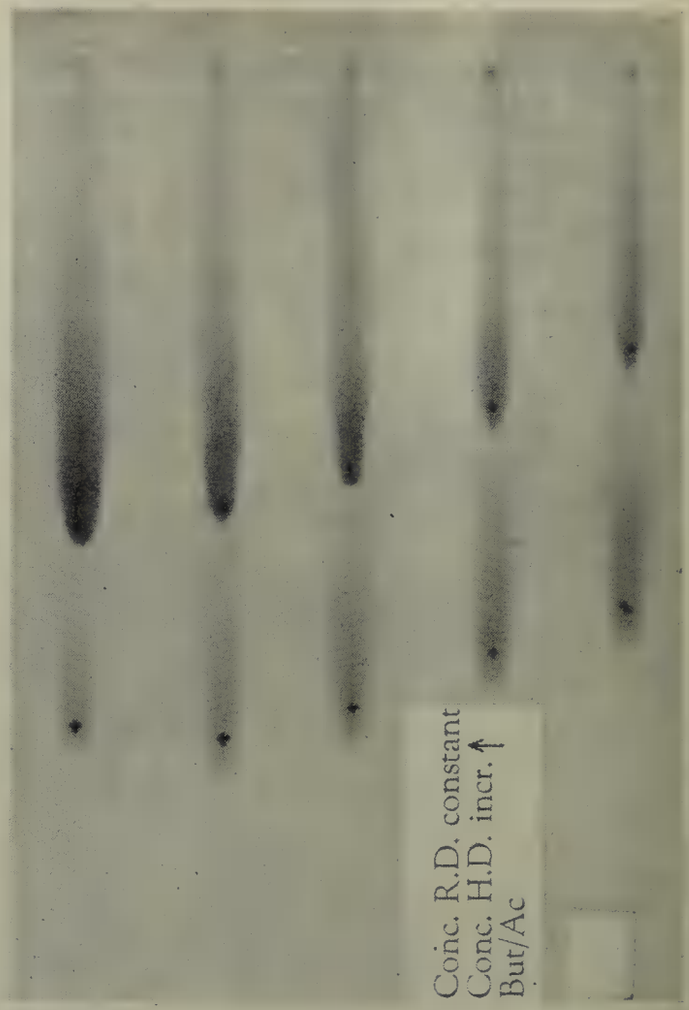


Fig. 2

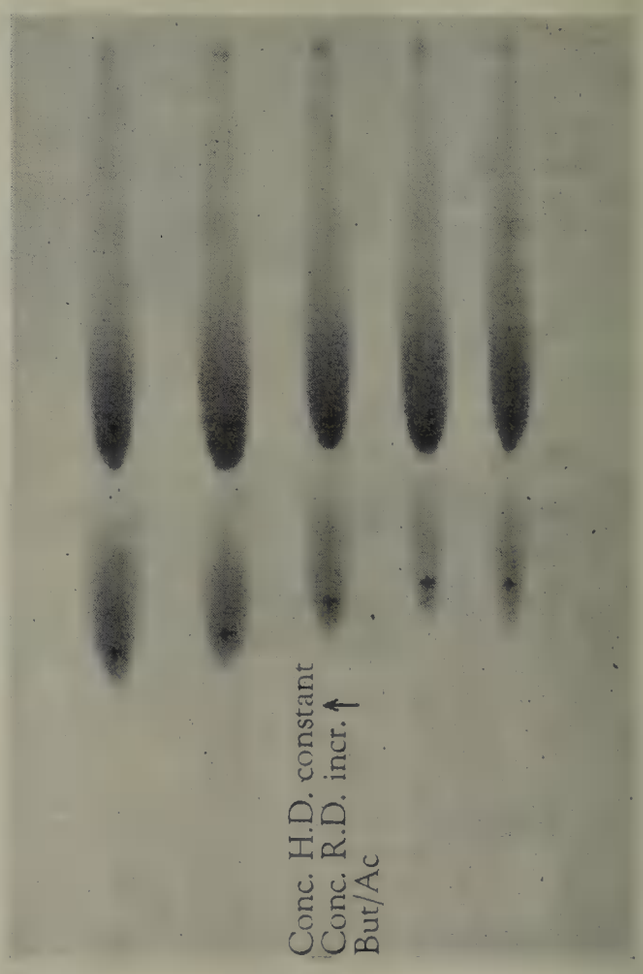


Fig. 3

been shown by several workers. It has been observed that the equilibrium adsorption of a particular dye from a solution containing a mixture of dyes is lower than from a solution containing this dye alone (Boulton, Delph, Fothergill and Morton[6]; also Neale and Stringfellow[7]). Such behaviour is considered to be due to the following causes: (1) Competition between the different dye anions for the available surface sites. (2) The effect of each dye in contributing to the surface charge on the fibre, and to the sodium ion concentration in it. (3) Interactions between the dyes in solutions.

In chromatography (1) is especially important since, under the conditions used, the support will be approaching saturation with dye. Applying all these factors to paper chromatography, one would expect the behaviour of a dye in mixtures to differ from that when it alone is present, especially in the case of dyes which are substantive to the support. It would therefore appear to be important that this possibility should be taken into account, when using paper chromatography, in order to detect and separate dyes in mixtures.

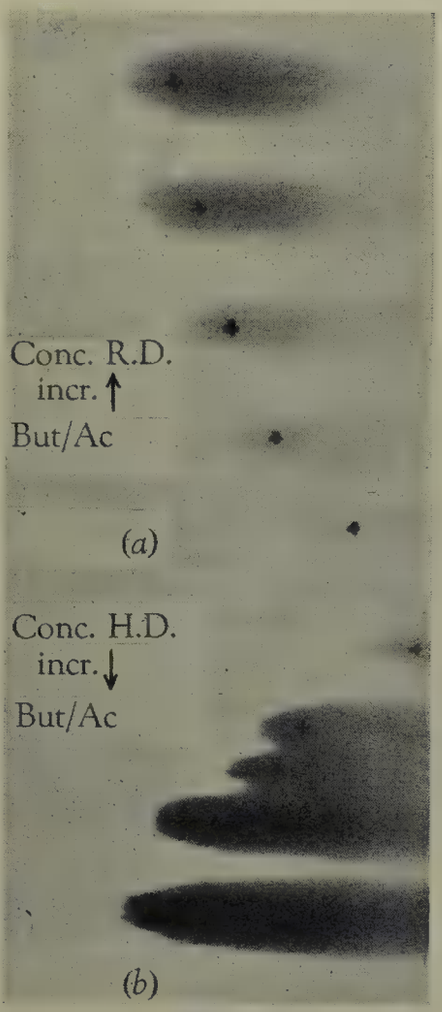


Fig. 4

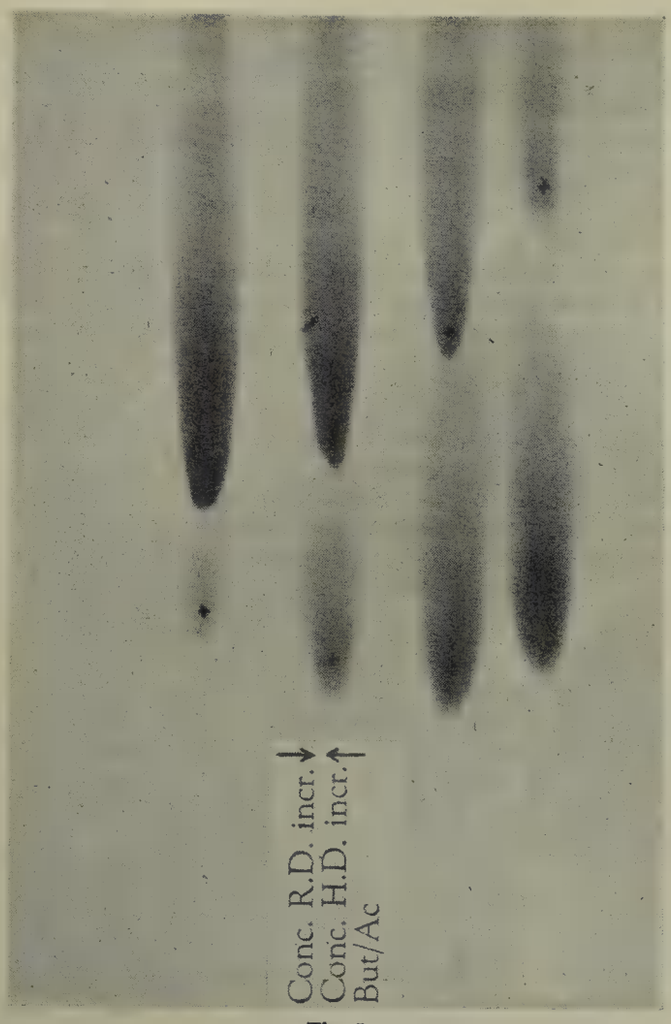


Fig. 5

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GEOCHEMISTRY OF ANDESITES AND THE GROWTH OF CONTINENTS

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THE growth of continents is one of the basic problems in geology. The rate of denudation is sufficient to erode continents to very low levels in periods brief (10–50 × 10^6 years) compared with the extent of geological time[1]. Accordingly, it is necessary to have a process by which material is added to the continents, not only to keep pace with the denudation, but also to allow for some growth. The chemical differences between continental crust and either oceanic crust or mantle demand that a chemical fractionation process operates as well as a simple addition of material.

Of the observable geological processes, basalt igneous activity is seen to add material from the mantle to the crust in both oceanic and continental regions. Granitic rocks and their associates seem to be derived from within the deep crust. In orogenic regions eruption of andesitic lavas and ash is the dominant volcanic activity and adds material to the surface at rates of perhaps 1 km^3 per year[2]. These orogenic zones are here interpreted as areas of active continental growth, as suggested by Wilson[28].

The andesites possess the following properties:

(1) They appear to be restricted to orogenic belts and are absent from oceanic areas.

(2) They are the most voluminous eruptive rock in these belts. Other rocks have occasionally been referred to as andesites (Table 1) but are excluded from this study.

Table 1. Nomenclature of Andesites and Analogous Rocks

	Some past usages		Present nomenclature
Calc-alkaline volcanic series	Andesite Orogenic andesite		Andesite
Alkali volcanic series	Andesine andesite Oligoclase basalt Trachyandesite (soda-rich) Trachyandesite (potash-rich)	Alkali andesites	Hawaiite (ref. 3) Mugearite (ref. 3) Benmoreite (ref. 4) Tristanite (ref. 4)
Tholeiitic volcanic series	Andesite Pigeonitic andesite Tholeiitic andesite		Icelandite (ref. 5)

They are developed in minor amounts in both continental and oceanic environments by fractional crystallization of basaltic magma[3–5] and are different petrographically and chemically from the true andesites.

(3) There is a common, but not universal association of more silica-rich rhyolites, ignimbrites and dacites.

(4) True basalts are often absent, or present in minor amounts; only occasionally are they abundant (for example, Japan).

(5) The andesites frequently contain glass, are typically porphyritic and the plagioclase exhibits reverse and oscillatory zoning indicating non-equilibrium conditions during crystallization. Present data suggest that they are characterized by hypersthene in the groundmass.

(6) They are broadly uniform in composition, although commonly associated with minor more basic andesites, containing large crystals of olivine and pyroxene.

(7) Their composition bears a general resemblance to previous estimates of that of the continental crust.

In Table 2, the average composition is given of well-described andesites from New Zealand[6,7], Saipan[8], California[9,10] and Japan[11,12]. Two recent estimates[13,14] of the composition of the continental crust are also given. The average composition of the common igneous rocks, granite and basalt is also given. Fig. 1 shows a comparison of the composition of the continental crust, averages of granite and basalt, and the range in composition of andesites (filled rectangles). The unfilled rectangles represent the composition of rarer basic andesites, possibly resulting from accumulation of olivine and pyroxene crystals.

It is apparent from Table 2 and Fig. 1 that the composition of andesites bears a general resemblance to that of the continents. The major anomaly is the potassium content, which is too low by a factor of two. Possible explanations of this will be discussed later. Because of the importance of the andesites, a programme of geochemical research has been started, and determinations of major and trace elements, and Sr isotopes has been carried out on samples from New Zealand and Japan.

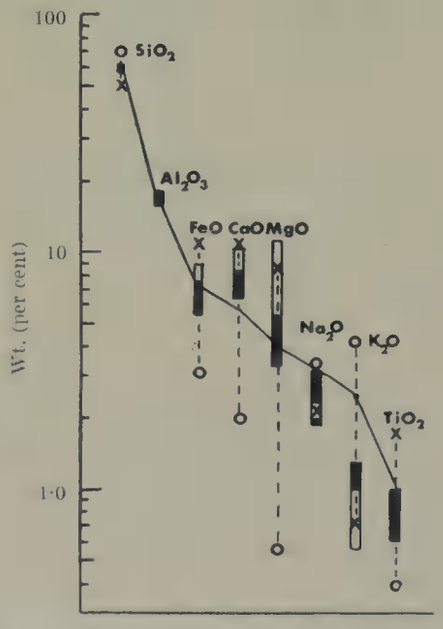

Fig. 1. Comparison of the major element composition of andesites, basalt, granite and continental crust. Filled rectangles, andesite; unfilled rectangles, basic andesite; ○, granite; ×, basalt; —, continental crust

Work is planned on samples from New Guinea and the Solomon Islands, Saipan in the Marianas arc, and the Cascade Province in western United States, in order to sample widely the circum-Pacific belt. Full details of samples, analytical methods and individual determinations will be given elsewhere.

The major and trace element data already available permit some tentative conclusions to be drawn. Data for the strontium isotopes will be presented separately[23]. The major elements were determined by conventional wet chemical methods, and the other elements by a combination of X-ray fluorescence, spectrochemical[19] and spark source mass spectrographic[20] techniques.

The trace element data are shown in Fig. 2. Average basalt and granite abundances are shown, with the estimation for the continental crust, based on a 1 : 1 mix of acid and basic rocks. Although there is a general similarity between the andesite composition and that of this average, the concentrations of many elements in andesites are lower than this crustal average. These include barium, rubidium, nickel, yttrium, lithium, thorium, caesium, uranium, tin and the rare earth elements. Except for nickel, these elements are all typically strongly concentrated upwards in the Earth since their large size, or valency, makes it difficult for them to enter the principal crystal phases in the mantle[21,22]. They are all typically concentrated in the last stages of fractionation, and resemble potassium among the major elements in this respect. K/Rb ratios for the common New Zealand andesites are 240, and 330 for the Japanese. The more basic andesites have ratios up to 340 for the New Zealand rocks, and 510 for the Japanese. The rare earth elements, although apparently lower in absolute concentrations, show similar patterns to those of well-mixed crustal sediments, when normalized to lanthanum −1·00. This is shown in Fig. 3 for the odd-numbered rare earths.

The trace element data provide limitations to the various theories of origin of andesites. These theories fall into three main categories: (1) Fractionation of basic magma, under oxidizing conditions. (2) Assimilation, contamination or mixing of acid and basic material. (3) Primary andesite magma developed by partial or complete melting of deep crustal or upper mantle material[29].

Volume relationships are generally not in favour of an origin by fractionation of basic magma. It is possible that the nickel content is too low for such an origin, but the trace element data do not exclude this possibility. The second alternative seems to be excluded by the present data. The low content (in andesites) of elements such as potassium, rubidium, uranium, thorium, rare earths, tin and lithium on the basis of any reasonable proportions of admixed granite rule out this mechanism. Preliminary results for strontium isotopic ratios support this conclusion[23].

Because of the serious limitations to the first process on geological grounds and to the second on geochemical arguments, the third process merits serious consideration. The relatively thin crust of about 30 km thickness present in typical orogenic areas[24] raises difficulties for an origin by fusion or partial fusion of deep crustal material. Temperatures to be expected at these depths are appropriate to the formation of granitic rocks[25], but may not be sufficient to form melts of andesitic composition. Experimental data bearing on this problem are lacking.

Table 2. Average Compositions of Andesite, Continental Crust and Common Rock Types

	Average andesite *A* (refs. 6–12)	Continental crust *B* (ref. 13)	Continental crust *C* (ref. 14)	Average granite *D* (refs. 15–18)	Average basalt *E* (refs. 15, 17)
SiO_2	60·1	59·4	60·3	71·2	48·9
Al_2O_3	17·2	15·6	15·6	14·7	15·7
FeO	6·1	7·1	7·2	3·2	10·7
MgO	3·5	4·2	3·9	0·6	8·7
CaO	7·1	6·6	5·8	2·0	10·8
Na_2O	3·3	3·1	3·2	3·5	2·3
K_2O	1·3	2·3	2·5	4·2	0·7
TiO_2	0·7	1·2	1·0	0·5	1·8

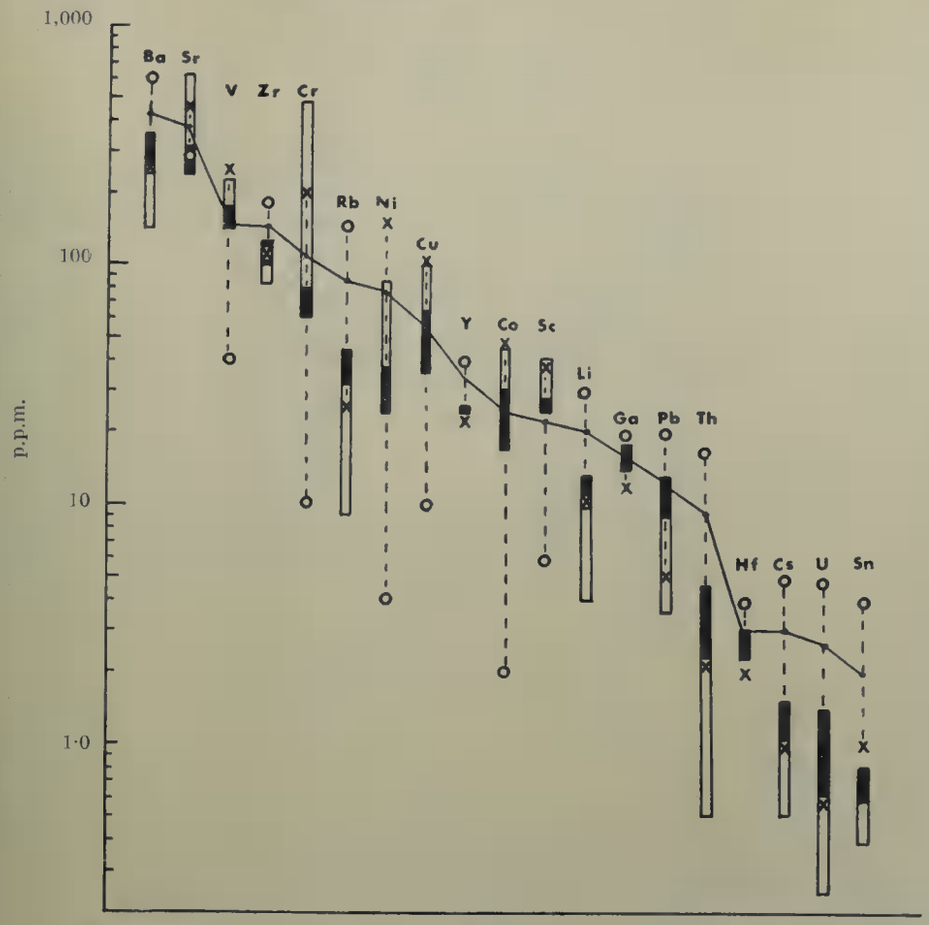

Fig. 2. Comparison of trace element composition of andesites, basalt, granite and continental crust. Filled rectangles, andesite; unfilled rectangles, basic andesite; ○, granite; ×, basalt; —, continental crust

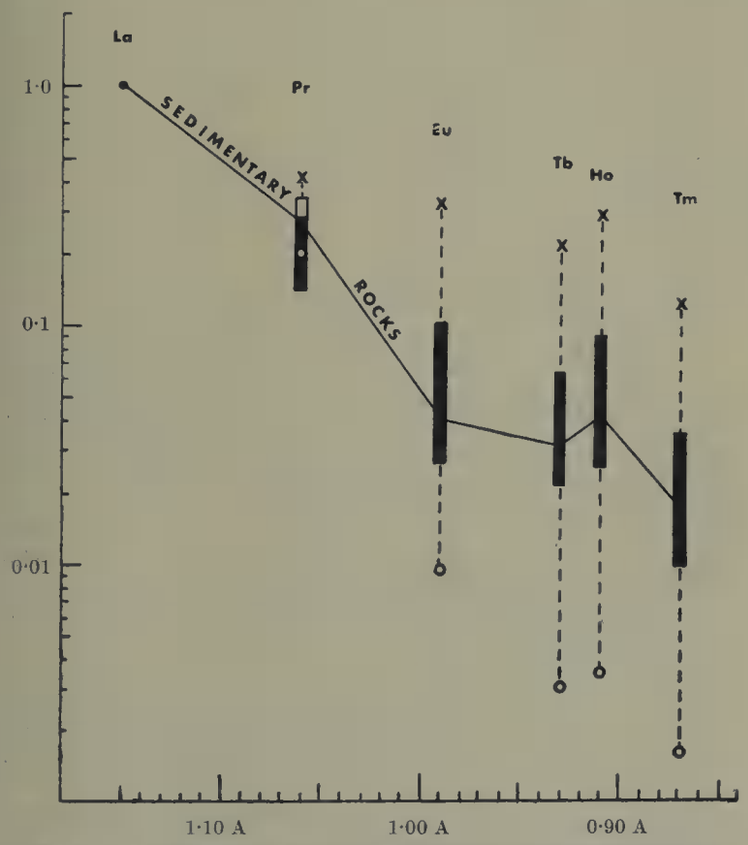

Fig. 3. Comparison of the odd-numbered rare earth abundance patterns, normalized to lanthanum, for andesites, basalt, granite and sedimentary rocks. Filled rectangles, andesite; unfilled rectangles, basic andesite; ○, granite; ×, basalt

If andesites are derived from the mantle, then the mantle beneath orogenic zones must differ significantly from that beneath continental shields or true oceanic areas, from which basalts are erupted. Recent workers in seismology[24,26] are agreed that the upper mantle beneath orogenic zones is characterized by an anomalously low velocity region for compressional (P) waves. This is interpreted as a region of lower than normal density for the mantle[26] immediately beneath orogenic regions. There is thus a correlation between andesite vulcanism and an anomalous low-density upper mantle. If andesites are derived from this low-density zone by partial melting, then they could make a major contribution to the growth of continents.

This hypothesis encounters the difficulty referred to earlier of a deficiency of potassium and related trace elements in andesites compared with present estimates of crustal composition. The following possibilities might explain this anomaly:

(1) Potassium and the related elements which are typically concentrated by fractionation processes might be overestimated in the continental crust. These elements could be concentrated in the upper and depleted in the lower crust so that sampling is overweighted in their favour. A lesser concentration of these elements in the crust would remove some difficulties for a chondritic Earth model[21]. Hence the composition of andesites may represent a more satisfactory overall average for the composition of the continental crust than previous estimates.

(2) The additional potassium, and other elements, could be provided by the acid volcanic rocks of rhyolitic composition commonly associated with andesites.

(3) Although we ourselves favour a philosophy based on the uniformitarian approach to geology[27], it is likely that the mantle has become depleted in potassium and related elements with time. Many of these elements should appear in the first products of partial melting. Thus recent andesites, studied here, could contain less of these elements than those of remote geological epochs. The composition of the continental crust would then represent the average of andesites erupted over a period of at least 3×10^9 years. Work is proceeding to test these several alternatives.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Interstellar Obscuration

An accurate extinction curve with a standard error of $\pm 0{\cdot}008^{m}$ which has been derived for a region in the direction of Cygnus (looking along the spiral arm) from spectrophotometric observations at the Royal Observatory, Edinburgh[1], shows a pronounced discontinuity in slope at 4300 Å. This feature as well as recent rocket observations[2] rule out the possibility that the interstellar dust is made up of iron or ice particles. Hoyle and Wickramasinghe[3] have suggested that graphite particles, formed on the surface of carbon stars, could be ejected into interstellar space by radiation pressure, and Wickramasinghe[4] finds that such carbon particles may become covered with ice mantles. The wave-length 4300 Å is close to that at which the refractive index of graphite particles begins to change, the extinction of graphite being remarkably close to a $1/\lambda$ law in the region $0{\cdot}8 \leqslant \lambda^{-1} \leqslant 2{\cdot}3$ and thereafter deviating from such a law more or less according to particle size[5]. Nandy and Wickramasinghe[6] have shown that the observed extinction curve for Cygnus can be well reproduced by the assumption of graphite cores with radii less than $\sim 0{\cdot}06\mu$ covered with ice mantles.

Similar observations have been made at this Observatory in the direction of Perseus (across the spiral arm)[7]. The extinction curve for Perseus shows that the change of slope occurs at the same wave-length, 4300 Å, as found for Cygnus stars; but that the slope of the ultra-violet part of the extinction curve relative to the blue part decreases by 30 per cent as compared with that in Cygnus (Fig. 1). The Perseus curve can be well represented by a Gaussian distribution of graphite particles with a dispersion of $\pm 0{\cdot}01\mu$ centred at $0{\cdot}06\mu$ (ref. 6).

The difference between the Cygnus and Perseus extinction laws could be explained if the interstellar matter were made up of particles covered with metallic impurities and aligned in a magnetic field[8]. The alignment properties of the particles would be different in Cygnus from those in Perseus because of the different angles of viewing, and the ratio of the slope of the ultra-violet part of the extinction curve to that in the blue would be expected to depend on the angle of viewing and the polarization of starlight per unit obscuration[9]. However, the work of Underhill and Walker[10], who examined the obscuration of stars in the southern sky, and of Johnson[11] shows that the variation of the law of obscuration is not dependent on the angle of viewing.

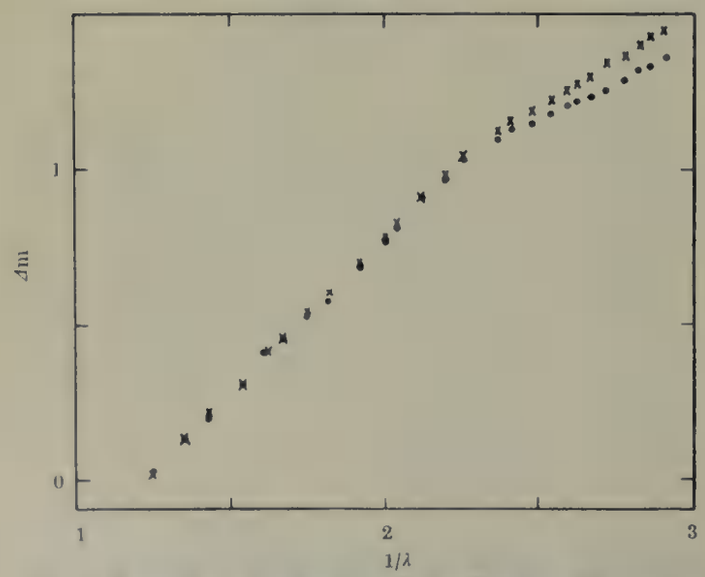

Fig. 1. Interstellar extinction curve in the direction of Cygnus and Perseus. Crosses denote results for Cygnus and filled circles results for Perseus

More light can be thrown on this matter by examining the obscuration of the star *HD* 23512 in the young Pleiades cluster. This relatively nearby cluster is in the Cygnus arm and is embedded in nebulosity. The polarization per unit obscuration of the star *HD* 23512 is the same as the average value in Perseus and about four times that in Cygnus.

This star has now been examined both here and by Mendoza[12]. The Edinburgh results agree well with those of Mendoza (Fig. 2). The extinction curve of the Pleiades star is found to be almost the same as that for stars in the Cygnus region, but significantly different from that in Perseus.

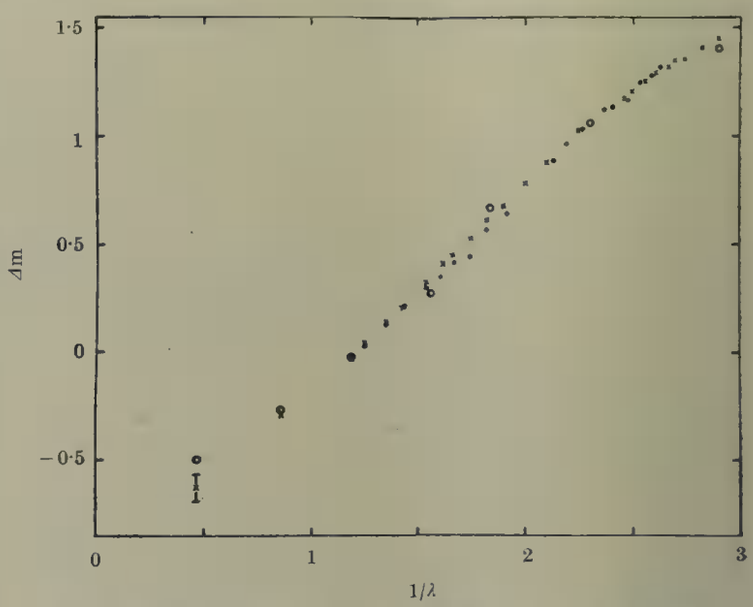

Fig. 2. Pleiades extinction curve as compared with that for Cygnus. The vertical line denotes the uncertainty of the point. ●, Pleiades (Edinburgh); ○, Pleiades (Mendoza); ×, Cygnus

The theoretical model which fits the Cygnus curve gives the value of the ratio of total to selective absorption $R = 3{\cdot}1$. The extrapolation of infra-red measurements of Mendoza for the Pleiades star gives $R = 3{\cdot}6$. An indirect measurement of R from measures of the line $H\gamma$ in the Pleiades stars gives $R = 3{\cdot}1$ (ref. 13).

The extinction of the Pleiades star does not show any correlation of the slope of the ultra-violet part of the extinction curve relative to the blue part with the polarization per unit obscuration. The variation in the extinction law cannot be explained therefore by differences in the alignment of the particles.

The space distribution of stars observed for the examination of extinction is illustrated in Fig. 3, which shows schematically the three spiral arms of the Galaxy. It is of interest to note that the Cygnus extinction curve is representative of the majority of stars in the Cygnus arm. In the direction of Perseus, the spiral arm can be easily reached, and the stars situated in a small region in the Perseus arm exhibit an extinction law different from that in Cygnus. Whether or not the Perseus law is representative of the Perseus arm in general has yet to be investigated. Few data exist as yet for stars in the inner arm. The difference between the Cygnus and Perseus extinction laws can be explained if it is assumed that the extinction in Perseus is due to graphite grains only, and that in

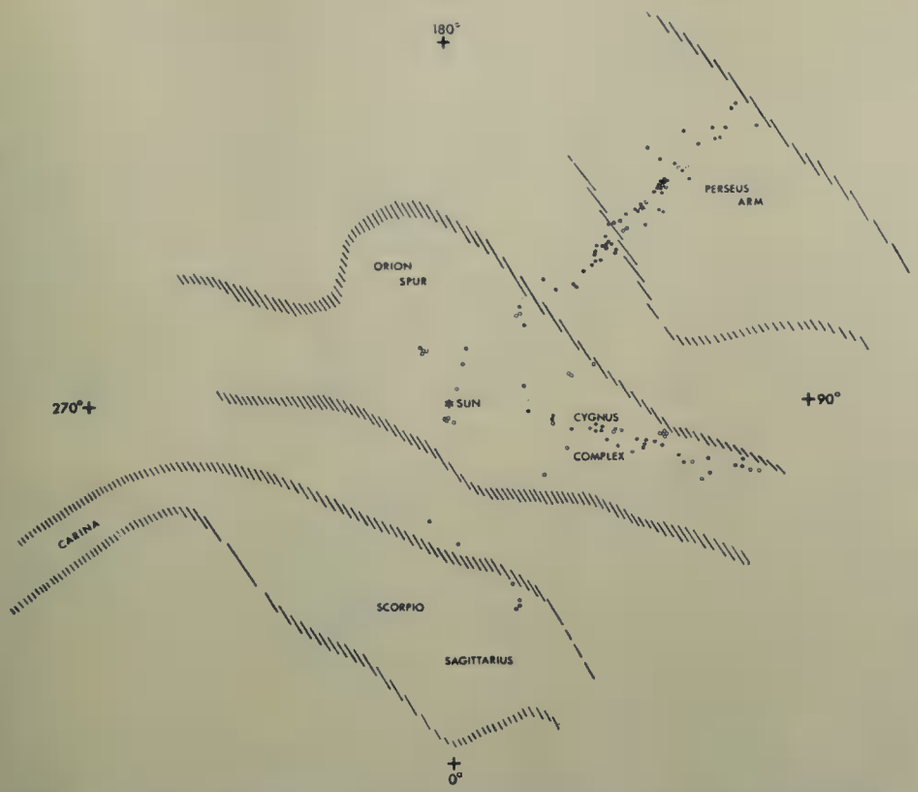

Fig. 3. Projection on the galactic plane of all stars observed for the study of interstellar extinction. Open circles refer to photoelectric measures, closed circles to photographic measures. The hatched lines represent the distribution of OB clusters, associations, and HII regions, and crosses represent directions of new galactic longitude on a circle 6 kiloparsecs in diameter

Cygnus to graphite cores with ice mantles. It appears therefore that there exists a regional variation in the composition of the interstellar dust particles which may be either a difference between the Cygnus and Perseus arm or a local variation within the Cygnus arm. The difference could be due to different physical conditions or to actual regional variations in chemical abundances. Further tests should come from the investigation of extinction in the Cassiopeia and Cepheus regions, which is now in progress here, and also from rocket observations.

I thank Mr. I. Nicolson and Mr. David Sugden for assistance with the reduction of the Pleiades plates.

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New Limits to the Angular Sizes of Some Quasars

In previous experiments at Jodrell Bank the angular sizes of discrete radio sources have been investigated by means of long baseline interferometers[1,2]. The highest resolving power used previously was obtained during observations at a wave-length $\lambda = 0{\cdot}73$ m with telescopes 180,000 wave-lengths apart (134 km). Four quasi-stellar and one unidentified source were found to be unresolved in those observations. Their angular sizes were thus shown to be smaller than 0·4 sec of arc. In a further attempt to resolve these sources another experiment has been carried out using the Mark I 250-ft. radio telescope at Jodrell Bank, and one of the 82-ft. radio telescopes operated by the Royal Radar Establishment, Malvern. The separation of these telescopes is 127 km, in a direction which is close to north–south. This interferometer worked on a wave-length $\lambda = 0{\cdot}21$ m, so that the maximum resolving power was more than three times greater than had been obtained in the previous observations. The effective resolving power changes as sources are observed in different directions, but its maximum value is greater than 600,000 wave-lengths for most sources. With this baseline the output fringe frequency produced as the radio source moves through the lobes of the interferometer pattern varies with hour angle from 0 to as much as 40 c/s. An almost identical frequency was produced continuously by a digital 'fringe speed machine' and this was subtracted from the interferometer output, so that the fringe patterns displayed on the chart recorder were normally slower than 0·5 cycles/min. A microwave link system was established between the two observatories, via two repeater stations. A new very-high-frequency phase-locking system was also developed, with equipment at each site.

During these observations, the five sources which had not been resolved previously (*3C* 119, 286, 287, *CTA* 21 and 102) all gave clear fringe patterns over a wide range of hour angles, though for only one source did the amplitude remain approximately constant. These sources are listed in column 1 of Table 1. Column 2 shows their catalogued[8] flux density, S, at $\lambda = 0{\cdot}21$ m. The observed fringe amplitudes were normalized by daily observations of the source *3C* 147, which was found to give clear fringe patterns at all hour angles. Corrections arising from various instrumental effects have been applied to these normalized values of fringe amplitude. It was found that when the sources were observed at elevations less than 15° these corrections were frequently greater than 20 per cent, and such observations have not been used. The corrected values of fringe amplitude have been calibrated by considering the maximum value observed for each source, which is shown in column 3 of Table 1. It was found that these maximum values are in an almost constant ratio to the total flux from each source, as shown in column 4, the mean value of these ratios being $14{\cdot}1 \pm 0{\cdot}9$.

A constant ratio in column 4 would be obtained if the minimum linear dimensions of all these sources were similar, and they were at comparable distances, and were partially resolved to the same extent by effective baselines between 500,000λ and 600,000λ. This is an improbable situation, and it seems more likely, as we assume, that each source was unresolved by the effective baseline at which the maximum value of fringe amplitude was

Table 1. Radio Sources Smaller than 0·1 Sec of Arc in at Least One Dimension

Source	Flux S at $\lambda = 0{\cdot}21$ m (flux units)*	Max. fringe amplitude, A, observed with tracking interferometer of aerial spacing 605,000λ (arbitrary units)	A/S
3C 119	$8{\cdot}5 \pm 0{\cdot}8$	125 ± 10	$14{\cdot}7 \pm 1{\cdot}8$
3C 286	$16{\cdot}1 \pm 0{\cdot}4$	245 ± 20	$15{\cdot}2 \pm 1{\cdot}5$
3C 287	$7{\cdot}6 \pm 1{\cdot}5$	95 ± 5	$12{\cdot}5 \pm 2{\cdot}5$
CTA 21	$8{\cdot}0 \pm 0{\cdot}8$	115 ± 10	$14{\cdot}4 \pm 2{\cdot}0$
CTA 102	$6{\cdot}6 \pm 1{\cdot}6$	95 ± 10	$14{\cdot}4 \pm 2{\cdot}0$
		Mean value	$14{\cdot}1 \pm 0{\cdot}9$

* One flux unit = 10^{-26} Wm^{-2}(c/s)$^{-1}$.

Table 2. Data on 3C 273 at 0·21 M

Total flux density (1962–3)	39·8 ± 2 flux units
Flux ratio component B : A (1962·9)	1·40
Therefore component A was then 16·5 and B,	23·3 flux units
Long baseline interferometer (1965·5)	
Two parts of one component gave	30 ± 2 flux units
Total power measurements (1965·6) $A + B$	46 ± 1·5 flux units
Provisional interpretation (1965·7)	

Component A is still 16·6 flux units, and component B is now 30 flux units. Flux ratio B : A now 1·84.

that, on the assumption that the radio galaxies have remained constant, the value of total flux from 3C 273 in 1965·6 was 46 ± 1·5 flux units. This is compatible with the other results if it is assumed that the interferometer observations refer to the component B, and that the flux of this component has now increased to 30 flux units, that is, by approximately 30 per cent in three years. It is not known which part of component B has

Table 3. Current Interpretations of the Radio Measurements of the Angular and Linear Dimensions of some Quasars

Source	Redshift z	Remarks on individual sources	Approximate linear dimensions (parsecs)	
			If redshifts cosmological*	If sources 'local' at about 10 Mpc
3C 273	0·158	Source has two components 19·5 sec apart in position angle 044°	Separation of A and B 32,000	950
		Component B, coincident with optical quasi-stellar object, has two parts, each smaller than 0·1 sec, separation probably 0·4 sec. At $\lambda = 0{\cdot}21$ m flux of component B has increased by approx. 30 per cent in 2·7 yr	Each part smaller than 170 separation probably 700	5 20
		Component A has dimensions 5 × 1·5 sec flux probably unchanged	8,500 × 2,500	240 × 70
3C 48	0·367	At $\lambda = 0{\cdot}73$ m, elliptical, 0·4 by < 0·3 sec†‡§	1,100 × ⩽900	20 × ⩽15
3C 47	0·425	At $\lambda = 0{\cdot}21$ m, this source has two components (ref. 9)		
		Separation 62 sec	⩾180,000	⩾3,000
		Each component ⩽10 sec, but little structure ⩽2 sec¶	< 30,000 but > 6,000	< 500 but > 100
3C 147	0·545	At $\lambda = 0{\cdot}73$ m, elliptical 0·6 by < 0·4 sec†‡§	2,000 × ⩽1,300	30 × ⩽20
3C 254	0·734	At $\lambda = 1{\cdot}89$ m, ≃6 sec. No structure measurements at any wave-length	≃21,000	≃300
3C 286	0·86	At $\lambda = 0{\cdot}21$ m, 0·12 by < 0·1 sec‡	420 × < 350	6 × < 5
3C 245	1·029	At $\lambda = 1{\cdot}89$ m, > 12 sec. No structure measurements at any wave-length¶	> 41,000 < 360	> 600 < 5
CTA 102	1·037	At $\lambda = 0{\cdot}21$ m, < 0·1 sec in all position angles		
3C 287	1·055	At $\lambda = 0{\cdot}21$ m, 0·14 × < 0·1 sec‡	500 × < 360	7·0 × < 5
3C 9	2·012	At $\lambda = 1{\cdot}89$ m. > 10 sec. No structure measurements at any wave-length¶	> 32,000	> 450

* These linear dimensions have been calculated for model universes (ref. 4) in which the acceleration parameter $q_0 = +1$. If $q_0 = 0$, these values must be multiplied by a factor $(1 + 0{\cdot}5z)$ which for these sources lies in the range 1–2·01.

† Results (ref. 7) of detailed analysis of observations at $\lambda = 0{\cdot}73$ m.

‡ None of the available evidence suggests that the source contains fine structure within this elliptical component, but further analyses or observations at even higher resolution could conceivably reveal a more complex structure, within these overall dimensions, as in the case of 3C 273B.

§ Fringe patterns corresponding to partial resolution of this source were recorded during the observations at $\lambda = 0{\cdot}21$ m described above. They have not yet been analysed in sufficient detail to improve significantly the earlier interpretation of the angular structure of this source.

¶ A weak source at $\lambda = 0{\cdot}73$ and $\lambda = 0{\cdot}21$ m. Even if it were unresolved, the signal-to-noise ratio would be poor under optimum conditions, and the fringe pattern might not have been recognized during those observations which were attempted.

observed. This means that each of these sources is smaller than 0·1 sec of arc in at least one direction. The source *CTA* 102 did not appear to be resolved at any hour angle when its elevation was greater than 15°, so that its angular size is shown to be less than 0·1 sec of arc in all position angles. Each of the other sources was partially resolved at some hour angle, and the corresponding maximum angular dimensions (assuming gaussian source models) were in the range 0·12–0·16 sec of arc, as shown in column 5 of Table 1.

The quasar 3C 273 was also observed and was found to give clear fringe patterns of unexpectedly large amplitude with one well-marked minimum near meridian transit. Observations of lunar occultations of this source show that it consists of two components, A and B, the centres of which are 19·5 sec apart in position angle 044°. If both these components had contributed to the fringe patterns observed at this baseline, a pronounced minimum would have been observed every 10–15 min at most hour angles. As these frequent minima were not observed, it follows that only one component of the source is small enough to give fringe patterns at this baseline, and that it, in turn, probably consists of two parts, which are each smaller than 0·1 sec of arc.

As only one minimum was observed, the angular separation of these parts and the position angle cannot, by inspection, be determined uniquely, though they may be obtained in due course by more detailed analyses. If it is assumed that this position angle is also 044°, the separation is of the order 0·4 sec of arc.

This interpretation of our observations, when calibrated with the factor derived from the sources discussed earlier, shows that the two parts of one component of 3C 273 are now giving a flux density not less than 30 ± 2 flux units. As may be seen in Table 2, this is significantly greater than the value of 23·3 flux units derived for the brighter component, B, from the ratio given by the occultation observations of 1962·9. The total flux from this source was therefore re-measured recently, with the Mark I telescope at Jodrell Bank, and compared with the radio galaxies 3C 348 and 353. These measurements show increased. This conclusion may be compared with the measurements reported by Dent[3] which show that at 8,000 Mc/s, where almost all the radiation comes from component B, the flux has increased by 40 per cent in 2·5 years.

We have summarized in Table 3 our current interpretations, based on these and earlier measurements, of the angular dimensions of the ten quasi-stellar sources the redshifts of which have been published[4,5]. Possible values of the linear dimensions of the radio-emitting regions of these sources are shown in columns 4 and 5. Those in column 4 have been calculated on the hypothesis that the redshifts of these sources arise from the general expansion of the Universe, and so correspond to distances greater than 600 Mpc. The values given in column 5 are calculated on the hypothesis suggested by Hoyle and Burbidge[6], that the emitting regions are at distances of order 10 Mpc, and their redshifts arise from very high intrinsic velocities of recession of the individual objects.

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RADIOPHYSICS

Scintillations on the Small Diameter Radio Sources

IRREGULARITIES in the solar wind appear to be responsible for the rapid scintillations on the radiation from small diameter radio sources[1], and for some of the fine structure on Jovian decametric bursts[2]. At the Arecibo Ionospheric Observatory the irregularities as well as the sources are being examined by their scintillations. Early results of this programme have been reported by Cohen and Drake[3]. The purpose of this communication is merely to report that the scintillation phenomenon has been seen over an extended range of frequencies and positions, and that it is very strong and persistent.

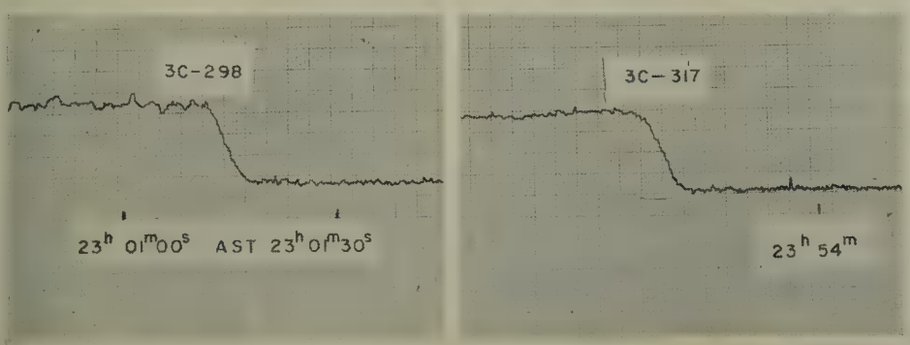

Fig. 1. 195 Mc/s records of 3*C*–298 and 3*C*–317, May 22, 1965. Successive on-off records are shown. Time constant 0·1

The scintillations are very strong when the source is very narrow and close to the Sun. For example, on May 23, 1965, *CTA*–21 was about 11° from the Sun. At 430 Mc/s the scintillations were very strong, with minima about 0·2 the mean value. As another example, on June 13, 1965, 3*C*–138 was about 7° from the Sun. Strong scintillations were observed on 195, 430 and 611 Mc/s. On all these frequencies the minima were about half the mean value. These observations were made with the 1,000-ft. spherical reflector, using point feeds giving beam-widths of 33′, 16′ and 14′ at 195, 430 and 611 Mc/s, respectively. Small diameter sources, such as *CTA*–21 and 3*C*–138 (ref. 4), can readily be found by their very strong scintillations, if they are close to the Sun.

The very narrow sources, furthermore, always scintillate, even when they are far from the Sun. Fig. 1 shows 195 Mc/s records of 3*C*–298 and 3*C*–317, taken on May 22, 1965, when 3*C*–298 was about 11 h east of the Sun. The two sources have approximately the same strength, as shown by the records; 3*C*–298 scintillates, but 3*C*–317 does not. The effect of scintillations shows up strongly in Figs. 2 and 3. Fig. 2 shows the unnormalized autocorrelation functions obtained from 5-min samples of the records, sampled 10 times per sec. The 3*C*–317 autocorrelation function has essentially the same strength and shape as those functions obtained when tracking the cold sky; it is an exponential with e^{-1} width equal to the output time constant of the radiometer. The autocorrelation function at zero lag is proportional to the variance of the fluctuating output signal, and so the root mean square fluctuation level for 3*C*–298 is twice that which would have been expected in the absence of scintillations. The scintillation index for 3*C*–298 is 0·041. (Index is defined as excess root mean square fluctuations divided by antenna temperature.)

Fig. 3 shows the power spectra of the radiometer output signals, obtained by computing the approximate Fourier transforms of the autocorrelation functions. The 3*C*–298 spectrum has excess energy only at low frequencies. This excess is the scintillation spectrum; it is roughly gaussian in shape, with an e^{-1} width of 0·55 ± 0·05 c/s. The peaks at 3·3 c/s are apparently due to aliasing.

A short radiometer time constant is required for scintillation observations, since the fluctuations may have periods of 1 sec or less. This results in a poor signal-to-noise ratio for weak sources. Digital processing, however, can greatly increase the visibility of the scintillations.

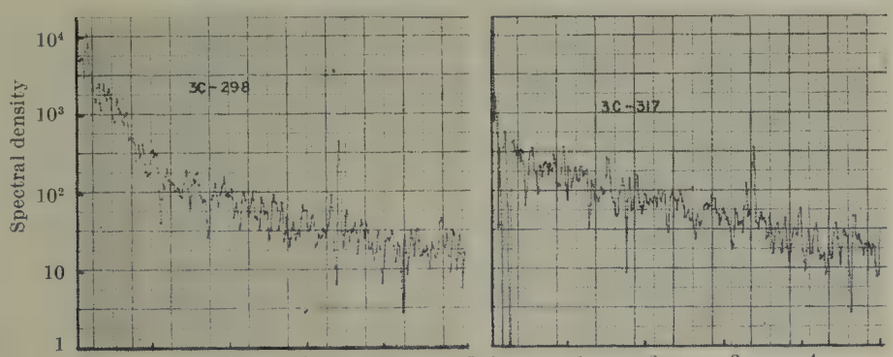

Fig. 3. Power spectra (in arbitrary units) for 3*C*–298 (scintillating) and 3*C*–317 (non-scintillating)

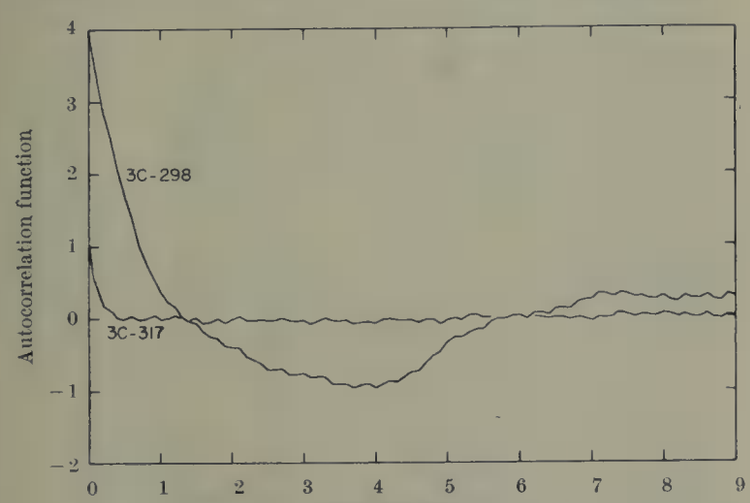

Fig. 2. Autocorrelation functions (in arbitrary units) for 3*C*–298 (scintillating) and 3*C*–317 (non-scintillating). Samples were taken 10 times per sec for 5 min; 90 lags are shown

I thank E. E. Salpeter for his advice, and Mrs. N. Earle and Mrs. S. Jack for assistance with the calculations. The Arecibo Ionospheric Observatory is operated by Cornell University with the support of the Advanced Research Projects Agency under a research contract with the Air Force Office of Scientific Research.

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PHYSICS

Combined Very High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars

In apparatus of the tetrahedral anvil or of the 'belt' type[1], where a solid-pressure transmitting medium such as pyrophyllite is used, the relation between load and pressure is fairly complicated[2]. The pressure calibration at room temperature is frequently made in terms of the usual resistance transitions in bismuth, thallium and barium[1]. There is no guarantee that such a calibration will be valid for the high-temperature range up to more than 1,000° C in which such apparatus is often used.

To check on this point, the tetrahedral anvil apparatus has been used to determine the melting points of zinc, aluminium, germanium and silicon as a function of the pressure inferred from a room temperature calibration. These results can then be compared with previous determinations[3–6] using piston and cylinder apparatus for which the temperature dependence of the load versus pressure calibration would be expected to be relatively small.

The NBS-type tetrahedral anvil apparatus used had 1·90-cm edge cemented (6 per cent cobalt) carbide anvils, and the 2·38-cm edge tetrahedra were made from pyrophyllite ('Alsil' grade). The experimental arrangement is shown in Fig. 1; the two alternative thermocouple positions shown gave identical results. The thermocouples used were chromel/alumel, and were of the sheathed 'Thermocoax' type with a sheath diameter of 0·5 mm: these were carried directly into the tetrahedron through an edge. No correction was applied for the effect of pressure on the thermal e.m.f. Melting was detected by simple thermal analysis, with the thermal e.m.f. displayed against a constant time base on a fast-acting XY recorder, since the cooling rate of the tetrahedral apparatus is rapid (400°/sec from 1,000° C).

The materials used in these experiments were 99·99 per cent pure or better and, to minimize possible contamination of the sample by its container, the following procedure was adopted. At a given anvil load, temperature was first raised slowly to ~0·7 of the expected melting point. Temperature was then increased rapidly (using the fast thermal response of the apparatus) by switching a suitable resistance from the primary of the heater power supply transformer. When melting was indicated by the appropriate 'arrest' on the rise curve, the heater supply was disconnected and the cooling curve taken. This ensured that the sample was heated in contact with its surroundings for a few seconds only, and X-ray microprobe analysis of selected specimens confirmed that no significant contamination had occurred during the heating cycle for any of the materials in tantalum containers. In view of previous experience[5], suggesting that molybdenum is a suitable container for aluminium, it is interesting to note that we have found evidence of considerable reaction between these metals at high pressures, especially at temperatures approaching 1,000° C, where depressions of the freezing point by as much as 50° C have been observed. X-ray microprobe analysis confirmed that a reaction had occurred in these cases with the formation of an intermetallic compound, probably $MoAl_2$.

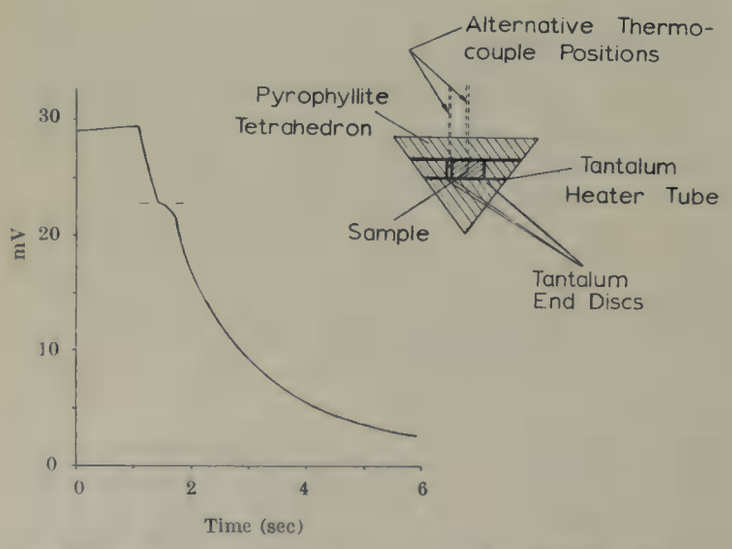

Fig. 1. Cooling curve of zinc specimen cooled from 720° C at 32 kbar, showing 'arrest' due to freezing. Inset shows tetrahedron arrangement

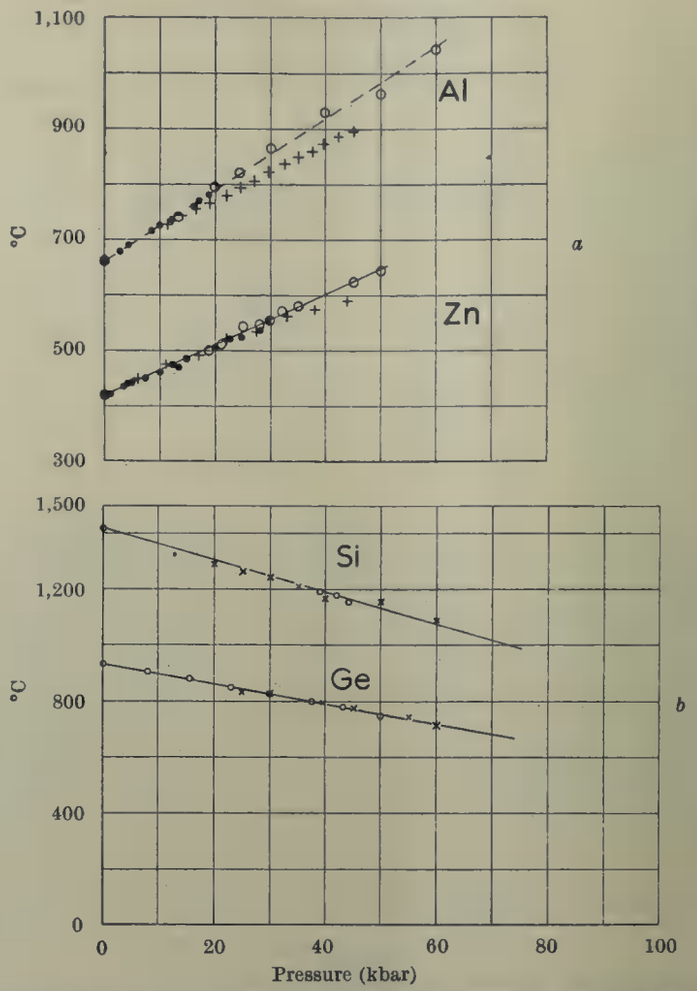

Fig. 2. Fusion temperatures as a function of pressure. (*a*) Zinc and aluminium; (*b*) germanium and silicon. ○, ref. 3; +, refs. 4 and 5; ×, ref. 6; ○, present work, pressures inferred from room temperature calibration

A typical arrest corresponding to solidification for a zinc specimen cooled from 730° C is shown in Fig. 1. In general, well-defined 'arrests' of this type were obtained during these experiments, and there was no evidence of supercooling despite the high cooling rates. Quite frequently 'arrests' began with a region of finite slope rather than an accurate horizontal; in these cases the point of inflexion was taken to indicate freezing. The maximum error of measurement in these experiments is about ± 5° C.

Fig. 2 shows the fusion curves to 60 kbar, pressures being inferred from a subsidiary room pressure calibration with a similarly sized test sample. All measurements were made during the loading cycle and the repeatability of pressures is in the range ± 1·5 kbar. These results are compared with two previous investigations: (1) Butuzov[3] used relatively large samples pressurized either in argon or pentane so that truly hydrostatic conditions were obtained. Melting temperature was determined by differential thermal analysis, and pressures by a manganin gauge with an accuracy of 0·1 kbar. (2) Using piston and cylinder apparatus with talc as the pressure-transmitting medium, Kennedy and Newton[4] have determined the fusion curve of zinc, Jayaraman, Klement, Newton and Kennedy[5] that of

aluminium, and Jayaraman, Klement and Kennedy[6] that of germanium and of silicon. Pressure was inferred from piston force and area, with a correction for piston friction, giving an accuracy of 1·0 kbar.

Fig. 2 shows a very satisfactory agreement between the fusion curves obtained in the present work and a linear extrapolation of those of Butuzov[3] for zinc and aluminium: further, that, in the range up to 60 kbar and 1,100° C, reasonable agreement is also obtained with the curves obtained in refs. 4 and 6 for zinc, silicon and germanium. Considering that the various determinations were made in widely differing types of apparatus, and in particular that Butuzov's conditions were truly hydrostatic, it is a reasonable conclusion that the effect of temperature on the load/pressure calibration curve for the tetrahedral apparatus is small, certainly less than ±3 kbar at 50 kbar and 1,000° C.

There is a significant difference, amounting to 8 kbar (or 50° C) at 40 kbar and 900° C, between the present results for aluminium and those of ref. 5. It is possible that at least some of this effect is due to the reaction discussed above between molten aluminium and the molybdenum containers used in the latter work.

In a recent determination of the phase diagrams of silicon and germanium, Bundy[7] used a modified 'belt' type of apparatus to produce pressures up to about 200 kbar and 1,000° C. With this apparatus a large pressure rise due to local heating at the specimen was inferred, which was roughly proportional to the product of the initial pressure and the temperature rise, and would amount to about 16 kbar at 50 kbar and 1,000° C. The compatibility of the present results with those of other workers implies that such a large effect is not encountered with the tetrahedral apparatus as used in this work.

We thank Miss A. Thake, who carried out many of the experiments, and L. Hailes for the microprobe analysis of specimens; we also thank C. H. L. Goodman for discussion.

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A New Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory

An absolute determination of the acceleration due to gravity at a site in the National Physical Laboratory has recently been completed. It is the first to be made by timing the symmetrical up-and-down motion of a body moving freely under gravity, and the uncertainty of the result is considerably less than that of any previous measurement.

If a body is timed as it crosses two horizontal planes with a vertical separation H, and if the time interval between crossings of the lower plane is T_1 and of the upper plane T_2, then:

$$g = \frac{8H}{T_1^2 - T_2^2}$$

A correction has to be applied for the decrease of gravity with height.

In the present experiment the planes were defined optically by pairs of horizontal slits and the moving object was a glass ball which focused one slit of a pair on the other when it was symmetrically between them. One slit was illuminated and a photomultiplier behind the other recorded the flash of light as the ball passed. The pairs of slits were incorporated in composite blocks of fused silica, the vertical distance (1 m) between the blocks at the upper and lower positions being measured by means of an interferometer.

The advantages of this method have been discussed recently[1] and it is sufficient to say that they have been fully borne out; in particular the measured acceleration is independent of air resistance.

The measured value of gravity, reduced to the British Fundamental Station in the National Physical Laboratory[2], is 981 181·77 mgal, with a standard deviation of 0·13 mgal (1 mgal = 10^{-5} m/sec^2).

The uncertainty comes mainly from microseismic disturbances. An earlier determination at the National Physical Laboratory with a reversible pendulum[3] gave a value of 981 183·2, *s.d.* 0·7 mgal at the British Fundamental Station. The difference of 1·4 mgal from the new result is just twice the standard deviation of Clark's value. Comparisons with other determinations will be discussed in the full account of the measurements which will be published later.

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Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid

The recent paper by Garton and Krasucki[1] is of considerable interest and reminded me of some of the work I undertook about five years ago[2]. I observed a similar phenomenon under different conditions. This brief note is to bring to notice some of my results obtained at that time, which serve as further evidence that gas bubbles and liquid drops always tend to elongate in the direction of the applied field in an insulating liquid, irrespective of whether the permittivity of the bubble or drop is larger or smaller than that of the surrounding liquid.

A glass test cell 5 in. × 3 in. × 2 in. was used, containing a pair of parallel brass plane electrodes of size 4 in. × 2 in. with a separation of 0·5 in. aligned horizontally. The test cell was fully filled with transformer oil, and an air bubble or a water drop could be introduced into the cell through two small holes in the top and the bottom of the cell. The pressure on the liquid was kept at atmospheric pressure and the temperature 20° C. The air bubble was injected through the bottom hole and it moved up through the centre of the liquid gap by buoyancy force, while the water drop was injected through the top hole and it moved down through the centre of the liquid gap by gravitation force. The 50-c/s a.c. field was first applied and a photograph was taken when the bubble reached the centre of the liquid gap. This method was different from that adopted by Garton and Krasucki, who deliberately eliminated the buoyancy force by rotating the test cell to maintain the bubble in a fixed position. However, their results and mine agree qualitatively. Fig. 1 shows an air bubble elongated at 22·5 kV and 28·5 kV though they were slightly distorted. Fig. 2 shows a water drop elongated and a large drop bursting into many droplets at 6 kV. The bubbles and drops in (*a*), (*b*) and (*c*) of Figs. 1 and 2 were not originally identical in size, since the device could not control their sizes accurately, But it can be seen that the greater the difference in permittivity between the bubble (or drop) and the insulating liquid, and the larger the original size of the bubble (or drop), the smaller is the electrical field required to cause elongation—as predicted by theory[1,2].

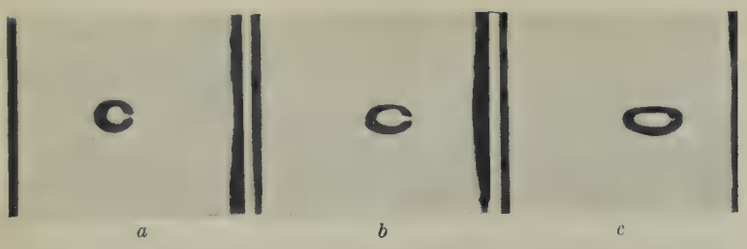

Fig. 1. An air-bubble elongated in the direction of the applied field in transformer oil. The 50-c/s a.c. voltage (root mean square) across a liquid gap of 0·5 in.: (*a*) $V = 0$; (*b*) $V = 22{\cdot}5$ kV; (*c*) $V = 28{\cdot}5$ kV

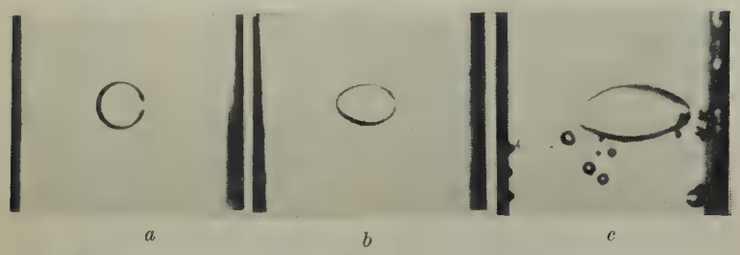

Fig. 2. A water drop elongated in the direction of the applied field in transformer oil. The 50-c/s a.c. voltage (root mean square) across a liquid gap of 0·5 in.: (*a*) $V = 0$; (*b*) $V = 6$ kV; (*c*) burst of a large water drop at $V = 6$ kV

It should be noted that the experimental conditions were somewhat different from the assumed conditions on which the theory was based. For example, the bubble size under experimental investigation was not so small compared with the liquid gap that the charge distribution on the electrodes before and after the introduction of a bubble was identical with the case for a field of fixed source distribution. This point must be borne in mind when comparing experimental results with theory. Furthermore, free charges inside or outside the bubble may also influence the deformation. Allan and Mason[3] have reported that under certain conditions the fluid drops were flattened into oblate spheroids in the field. This phenomenon may possibly be due to the attractive force between positive and negative charges accumulated on the bubble surface, which, so far, has been ignored in the theory. More work, both theoretical and experimental, is necessary in order to clarify many points which are still not understood.

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GEOPHYSICS

Variation according to Latitude of the Electron Content of the Ionosphere near the Auroral Zone

Some evidence has been obtained from single-frequency Faraday measurements using the satellite 1963–49*C*, reported by Liszka and Taylor[1], that the sub-satellite electron content, n_t, of the ionosphere at high latitudes may consist of two components. The first component, which decreases gradually with increasing latitude, is probably produced by ultra-violet radiation from the Sun; the second, irregular, component seems to dominate at latitudes greater than 65° N. It was suggested that the latter could either be produced by low-energy corpuscular radiation, or that it could be a manifestation of transport processes. Some more accurate measurements of the electron content at high latitudes, obtained from differential Faraday observations of the multifrequency beacon satellite *S*–66 (1964–64*A*), now show that the first explanation of the irregular component seems more plausible.

The electron contents have been calculated using data from 40 and 41 Mc/s records of *S*–66 made at Kiruna, Sweden (67·8° N., 20·4° E.), during the first two months of the satellite's life. The method was the same as that used for the reduction of two-frequency Moon radar data[2]. n_t was calculated at each Faraday null time and plotted as a function of sub-ionospheric geocentric latitude. Some of the resulting curves are shown in Figs. 1 and 2; those corresponding to a time of day shortly before local noon are given in Fig. 1 and those to a time shortly after local midnight in Fig. 2. All these results refer to the second half of November 1964. It can be seen that near noon n_t decreases with latitude; small irregularities often occur at latitudes of about 65° N. Night-time curves, particularly those from 03–04 local time, show a gradual decrease of n_t up to about 60° N., followed by an increase up to values at least as great as those observed at 50° N.

It may also be noted that the electron contents shown here are, on average, lower than those obtained from single-frequency records by assuming the effective thickness of ionosphere to be 350 km (ref. 1). Equivalent slab thicknesses were calculated using simultaneous values of maximum electron density, N_{max}, obtained from the equation:

$$N_{max}(\mathrm{e/cm^3}) = 1{\cdot}24 \times 10^4 (f_0F2)^2$$

where f_0F2 is the ordinary wave critical frequency in Mc/s. For this purpose f_0F2 values from six Scandinavian stations, covering an interval of 10 degrees of latitude, were used. The results show that at high latitudes the effective thickness is much less than 350 km during the daytime, and sometimes less than 200 km. However, when the second component of n_t was present, effective thicknesses a few times larger than 350 km were obtained. As an example, effective thicknesses obtained for one of the transits shown in Fig. 2, transit No. 658, are given in Table 1 (Nov. 27, 1964, 0200 local time).

Table 1

Station	Geographic latitude (° N.)	f_0F2 (Mc/s)	Effective thickness (km)
Sodankylä	67·4	2·0	650
Lycksele	64·7	1·9	413
Uppsala	59·8	2·0	222

An examination of f_0F2 values shows that in many cases during the night-time, N_{max} changes with latitude in the same way as n_t, that is, it shows a definite minimum value. As an example, N_{max} values from 03 to 04 local time of November 22 are shown in Fig. 3. This phenomenon may also be found during the early morning hours on averaged world contours of f_0F2 published by Martyn[3].

Any transport of ionization is almost certainly directed along the magnetic field lines, that is, nearly vertically, and it seems unlikely that the large increase of the

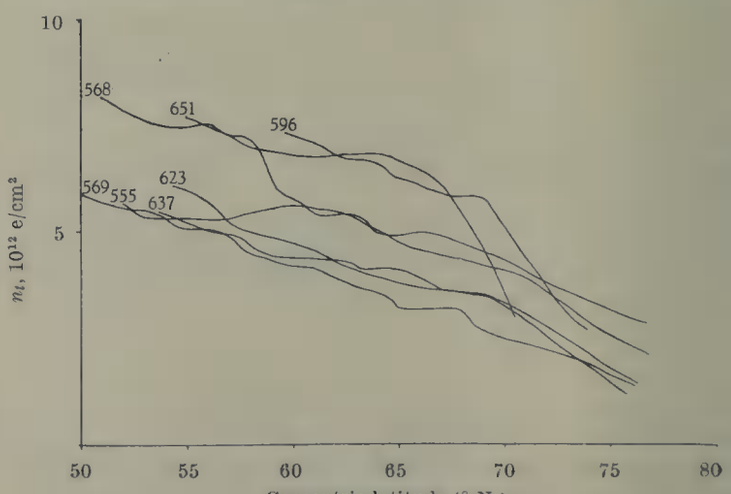

Fig. 1. Electron contents for a number of satellite transits shortly before local noon during the second half of November 1964 plotted as a function of geocentric latitude

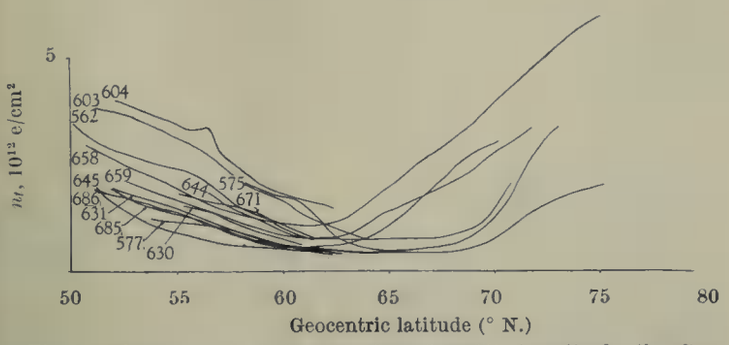

Fig. 2. Electron contents for a number of satellite transits shortly after local midnight during the second half of November 1964 plotted as a function of geocentric latitude

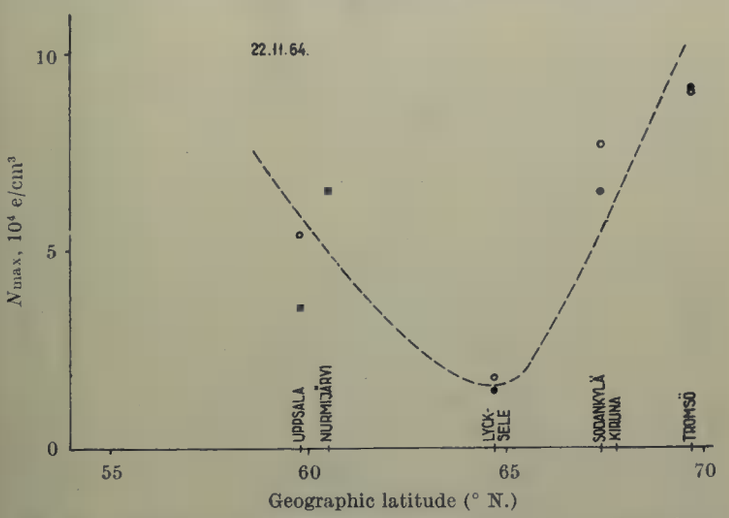

Fig. 3. Electron densities at the maximum of the *F*-layer, N_{max}, from 03 (○) to 04 (●) local time on November 22, 1964, plotted as a function of the geographic latitudes of the ionosonde stations

electron content up to the satellite altitude (1,000 km) can be due to this cause. It would mean that there are at least as many electrons above 1,000 km as below; electrons which can, moreover, in certain conditions move downwards. There is at present no evidence for the existence of such a reservoir of ionization in the upper *F*-region, though there are few relevant high-latitude observations. Thus it seems more reasonable to attribute the increase of n_t to low energy corpuscular radiation, possibly electrons, producing additional ionization in the ionosphere at auroral latitudes. Their energy must be less than 1 keV since electrons with higher energy reach altitudes below 180 km without ionizing the *F*-layer[4].

Further investigation of electron contents at auroral latitudes is needed before any definite conclusions can be drawn. It is already clear that the *S*-66 satellite is an excellent instrument in this type of investigation.

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GEOLOGY

Holothurian Sclerites from the Speeton Clay

During an examination of the Foraminifera from the Lower Cretaceous clays of Speeton, Yorkshire, a number of samples were found to contain holothurian sclerites. This was an unexpected occurrence, as Frizzell and Exline[1] have pointed out that virtually no work has been published on sclerites from strata of Cretaceous age. The only previous records of Cretaceous holothurian sclerites are *Calcligula* (?) *huckei* Frizzell from the Gault of Pomerania, *Theelia rotula* (Egger) from Germany and *Hemisphaeranthos frankei* (Müller) from the Turonian of Germany. '*Chirodota*' from the Hauterivian of France were noted by Deprat[2], and Wetzel[3] obtained sclerites from a Baltic flint which Deflandre-Rigaud[4] identified as *Myriotrochites elegans* (Schlumberger), *Chiridotites atavus* (Waagen) and *Chiridotites* cf. *ingens* (Joshua). More recently Kemper[5] recorded 'Holothurienreste' from the Hauterivian of Germany, though he did not describe them.

A systematic search for holothurian spicules in more than two hundred micropalaeontological residues of the Speeton clay, mainly from the type locality but also from borehole material, revealed several horizons which contained a large number of holothurian remains. All were obtained from strata of Hauterivian and Barremian age.

The sclerites from Speeton belong to four families, Achistridae, Theeliidae, Stichopitidae and Priscopedatidae; all are well preserved and free from matrix and secondary growth. Of particular interest in the Barremian clays is the occurrence of the Family Achistridae, which is represented by the species *Achistrum* (*Cancellrum*) *monochordata* Hodson, Harris and Lawson. This is a sclerite in the form of a hook, with a terminal loop which is crossed by a single cross-bar. Forty-one examples of this species have so far been found. With this record of *Achistrum* (*Cancellrum*) *monochordata* the range of the Family Achistridae, previously regarded as being from the Devonian (?) to the Jurassic, can now be extended to include the Lower Cretaceous.

It is interesting to note that *A. monochordata* is a common form species in the Oxfordian[6,7], though the specimens from the Barremian are generally smaller than those from the Jurassic. The widths of the terminal loop in the specimens from Speeton range from 0·090 mm to 0·144 mm, while those from the Oxfordian range from 0·162 mm to 0·324 mm.

So far as I am aware, holothurian sclerites have not previously been recorded from the Cretaceous of Britain.

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CHEMISTRY

Polyamide Layer Chromatography of DNP Amino-acids

Methods for the identification of DNP amino-acids by paper or thin-layer chromatography were used to determine the *N*-terminal group in polypeptide structures. Recent developments in silica-gel thin-layer chromatography have made possible the rapid identification of amino-acids in biological mixtures[1].

The separation of various DNP amino-acids on a polyamide column was developed by Steuerle and Hille[2] and by Grassmann *et al.*[3], but no practical method for separating micro samples by polyamide thin-layer chromatography has been developed.

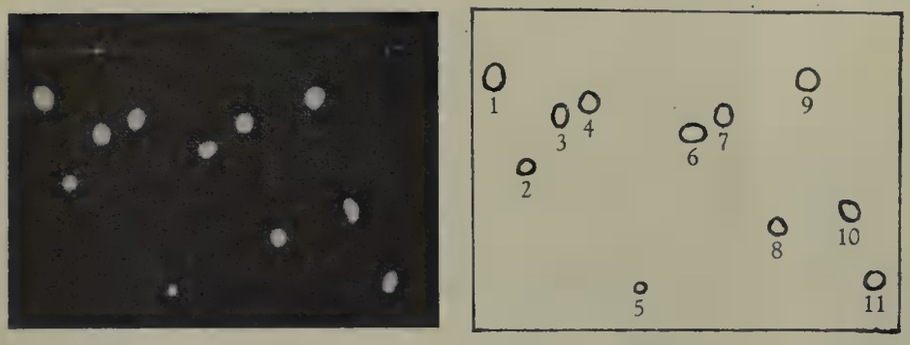
The polyamide layer (Chen-Hsin-Tang Chemicals Co., No. 75, Section I, HanKou St., Taipei, Taiwan), according to Wang[4], gave better results and durable polyamide layer. We have applied this polyamide layer to several

Table 1

	A 4 h	*B* 1·5 h	*C* 1·5 h	*D* 1·5 h	*E* 1 h
ε-DNP-Lys-HCl	0·72	0·14	0·56	0·91	0·95
DNP-DL-Ser	0·37	0·41	0·22	0·33	0·63
DNP-DL-Leu	0·80	0·81	0·55	0·33	0·77
DNP-L-Asp	0·27	0·31	0·17	0·25	0·61
DNP-DL-Met	0·59	0·72	0·37	0·21	0·69
DNP-Ala	0·58	0·63	0·35	0·34	0·70
bis-DNP-His	0·60	0·12	Tailing	Tailing	0·79
DNP-DL-Val	0·80	0·74	0·56	0·36	0·76
bis-DNP-Lys	0·29	0·69	0·18	Tailing	0·44
DNP-Thr	0·50	0·51	0·32	0·34	0·70
DNP-Isoleu	0·85	0·82	0·57	0·28	0·77

A, *n*-Butanol–acetic acid (9 : 1); *B*, 2-butanone–chloroform–formic acid (3 : 7 : 1); *C*, ethanol–water (63 : 37); *D*, dimethylformamide–acetic acid–water–ethanol (5 : 10 : 30 : 20); *E*, methanol–formic acid (9 : 1).

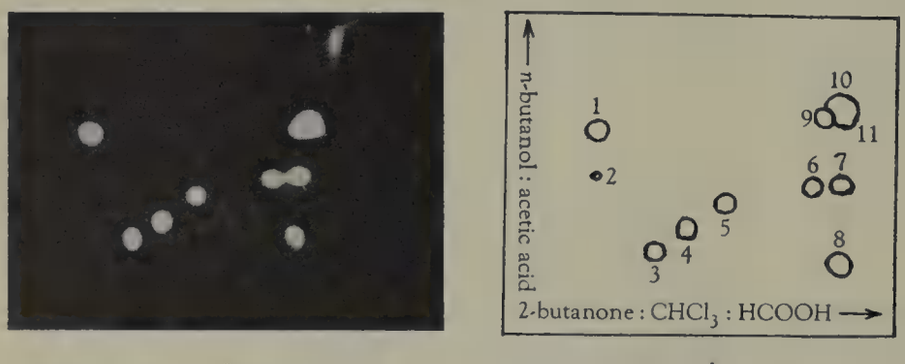
DNP amino-acids. Five solvent systems: solvent *A*, *n*-butanol–acetic acid (9 : 1); *B*, 2-butanone–chloroform–formic acid (3 : 7 : 1); *C*, ethanol–water (63 : 37); *D*, dimethylformamide–acetic acid–water–ethanol (5 : 10 : 30 : 20); *E*, methanol–formic acid (9 : 1), were excellent for one-dimension chromatography. The R_F values and the time required for plate development are shown in Table 1, and Fig. 1*a* shows a typical ultra-violet contact photograph of these chromatograms.



a *b*

Fig. 1. Plate: 15 cm × 15 cm; solvent system: 2-butanone–$CHCl_3$–HCOOH (3 : 7 : 1) 13 cm. Key: 1, DNP-Isoleu; 2, DNP–Thr; 3, *bis*-DNP–Lys; 4, DNP–DL-Val; 5, *bis*-DNP–His; 6, DNP–Ala; 7, DNP–DL-Met; 8, DNP–H–Asp; 9, DNP–DL-Leu; 10, DNP–DL-Ser; 11, ε-DNP–Lys–HCl

In both solvent systems *C* and *D*, the times required for development are faster than solvent system *A*, but some of the DNP amino-acids give tailing spots. Solvent *B* exhibits a completely different tendency in R_F values. The similar effect of chlorinated hydrocarbon was also observed in phenols[4]. The solvent system *D* is the same as that used by Grassmann *et al.*[3] for column chromatography.



a *b*

Fig. 2. Plate: 15 cm × 15 cm; solvent system: first, BuOH–AcOH (9 : 1) 12 cm; second, 2-butanone–$CHCl_3$–HCOOH (3 : 7 : 1) 13 cm. Key: 1, ε-DNP–Lys–HCl; 2, *bis*-DNP–His; 3, DNP–H–Asp; 4, DNP–DL-Ser; 5, DNP–Thr; 6, DNP–Ala; 7, DNP–DL-Met; 8, *bis*-DNP–lys; 9, DNP–DL-val; 10, DNP–Isoleu; 11, DNP–DL-Leu

The combination of solvent systems *A* and *B* in two-dimensional development was also tried. This is shown in Fig. 2*a* and *b*. Leucine and isoleucine cannot be separated clearly by these systems because these two are too closely related in structure.

The polyamide layer gives less diffused spots and better separation than silica-gel thin-layer chromatography. This durable polyamide layer with high resolution might be a promising method for the identification of DNP amino-acids.

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Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats

Previous workers have shown that sera of various mammals are capable of binding plutonium. Hunts and Barron[1] prepared serum from dogs which had been intravenously injected with a hexavalent plutonium citrate complex. Electrophoresis of the serum in a Tiselius type of apparatus showed that the β-globulin fraction combines with the plutonium. Beliayev[2], using paper electrophoretic methods, showed that plutonium is bound principally to the globulin fractions. The serum used in this latter piece of work was obtained from rats 24 h after intraperitoneal injection of a tetravalent plutonium citrate complex. The results obtained by Beliayev were not very reproducible, due possibly to buffer salt interactions and the influence of the electric field. Accordingly we have measured the distribution of intravenously injected tetravalent plutonium in the blood serum of the rat. The results presented here show that in blood obtained from the rat within 0·5 h of injection, the plutonium is bound to the transferrin proteins.

The serum used for this work was prepared by Dr. D. M. Taylor of the Institute of Cancer Research, Sutton, Surrey. The plutonium stock was $Pu(NO_3)_4$ in nitric acid. Immediately before injection of about 0·1 μc., the sample was diluted with water. The elapsed time between dilution of the sample and its injection into the rat was short. Consequently the principal plutonium species injected into the rat was the tetravalent one. The rat was killed within 30 min of the injection. The blood was allowed to clot at room temperature for 2 h, stood overnight at 4° C, and then centrifuged. The supernate was stored at −20° C until required.

The blood proteins were separated into three fractions by the relatively mild method of gel-filtration, and further resolved by ion exchange as described by Gelotte, Flodin and Killander[3]. The distribution of the serum proteins in the eluates was determined spectrophotometrically at 254 mμ. The relative plutonium content of protein samples was determined by counting the delayed neutron emission following neutron irradiation. Delayed neutron emission induced by thermal neutron irradiation of fissile nuclides is a highly specific property, and has been used by Amiel[4] as an analytical method for the determination of uranium-235. The plutonium supplied by the Radiochemical Centre, Amersham, is free from other fissile elements, hence Amiel's method was used as a simple and rapid method of analysis.

Plutonium was found to be present in the albumin/transferrin fraction only, following protein resolution by

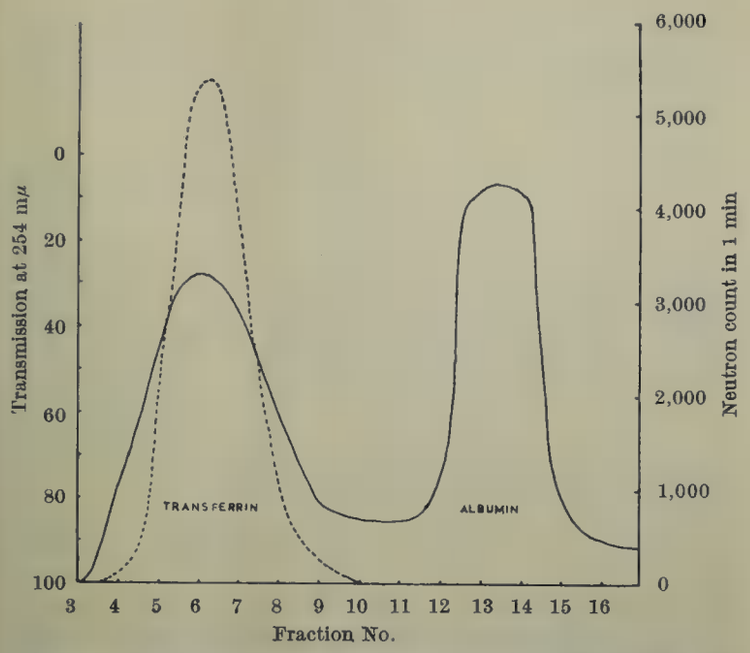

Fig. 1. Chromatography of the transferrin/albumin fraction on a column of DEAE-'Sephadex *A*-50'. Elution was started with 0·1 M 2-amino 2-hydroxymethylpropane 1 : 3 diol (*tris*) and its hydrochloride in 0·1 M sodium chloride, *p*H 8·0, followed by a gradient of 0·1 M–2·0 M NaCl in 0·1 M *tris*-HCl. The fraction volumes were about 3·5 ml. —, Transmission; – – – –, radioactivity

'Sephadex *G*-200' gel-filtration. Resolution of the albumin/transferrin fraction into its components by DEAE-'Sephadex *A*-50' and subsequent delayed neutron counting showed plutonium to be present in the transferrin fraction only as depicted in Fig. 1. Additionally the protein fractions were characterized by electrophoresis on cellulose acetate strips, using veronal buffer at *p*H 8·6 and staining with Ponceau *S* (ref. 5). Autoradiography of the unstained electrophoresis strips demonstrated that the plutonium migrates at the same rate as the transferrin.

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Far-infra-red Spectra of some Square-planar Ions

There are three different assignments of the infra-red-active modes of K_2PtCl_4 in the literature[1–3]. One was based on a low-resolution spectrum[1] and is thus misleading. The other two authors differed in their assignment of ν_2 and ν_7, but their data are in good agreement except that Mathieu *et al.*[3] reported a band at 160 cm^{-1} which was not found by either Sacconi *et al.*[2] or by ourselves. We have observed the spectrum of K_2PtCl_4 to 40 cm^{-1} and support Sacconi's results and assignment for the following reasons.

(1) The site symmetry of $[PtCl_4]^{2-}$ in the potassium salt is the same as that of the free ion. It is thus difficult to account for the triplet nature attributed to ν_7 by Mathieu *et al.* (2) The band near 110 cm^{-1} assigned by Mathieu *et al.* to ν_2 is absent in the spectrum of Magnus's green salt, and can be assigned to a translational lattice mode of the potassium ions, three of which should be infra-red-active. We find additional lattice modes at 87·5 and 59 cm^{-1}, which may also be associated with the potassium ions.

We have made the first observations of ν_2 and ν_7 for K_2PdX_4 (X = Cl and Br), of ν_6 for $KAuI_4$, and of the complete far-infra-red spectra of $[M(NH_3)_4]$ $[M'Cl_4]$ [M, M' = Pd, Pt]. Our assignments for the complex ions in these compounds are supported by the band width considerations: those bands assigned to a_{2u} modes are sharp and narrow, while those assigned to e_u modes are at least twice as broad. A similar band width effect is seen in ν(CO) bands for complexes of the type $M(CO)_3$ (π-arene).

Table 1. Infra-red-active Frequencies (cm^{-1}) of some Square-planar Ions

	ν_2 (a_{2u})	ν_6 (e_u)	ν_7 (e_u)	Other bands
K_2PtCl_4	174·5	326	194	108·5, 87·5, 59
K_2PdCl_4	168	332	190·5	111, 90
K_2PdBr_4	106	254	136	80·5
$[Pt(NH_3)_4]^{2+}$	141·5	—	234	266, 82·5, 74
$[PtCl_4]^{2-}$	175	310	198	
$[Pd(NH_3)_4]^{2+}$	144	—	226	263, 93, 80·5
$[PtCl_4]^{2-}$	174	321	187·5	
$[Pt(NH_3)_4]^{2+}$	136·5	—	245	364, 288, 93, 83
$[PdCl_4]^{2-}$	163·5	332·5	224	
$KAuI_4$	—	190·5	—	—

All the ions in the complexes $[M(NH_3)_4]$ $[M'Cl_4]$ have site symmetry C_4. There should not therefore be any splitting of existing degeneracies, but the Raman-active ν_1 in the free ions should become infra-red-active. We have not observed it, but we do find a weak-medium band at 266 cm^{-1} (M = M' = Pt), 263 cm^{-1} (M = Pd, M' = Pt) or 288 cm^{-1} (M = Pt, M' = Pd). This is very low for ν_1 for any of the complex ions and we tentatively assign it to a combination of ν_7 of the ammine complex ions with a lattice mode. There is no evidence that the metal-metal interaction in Magnus's green salt has any significant effect on the infra-red spectra of the complex ions, as compared with K_2PtCl_4 and $[Pt(NH_3)_4]Cl_2$. In particular, the a_{2u} out-of-plane deformation bands of $[PtCl_4]^{2-}$ are constant in both frequency and intensity.

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Methane-rich Perchloric Acid Flames

The study of flames supported by the vapour of 72 per cent perchloric acid[1,2] has been continued by an investigation into methane-rich premixed flames of perchloric acid. Perchloric acid flames have already been shown to have no effective lower flammability limit since a decomposition flame of the acid vapour alone can be stabilized[2]. It has now been found that mixtures containing up to at least 84 per cent methane in perchloric acid are flammable; this corresponds to 20 times as much methane as in a stoichiometric mixture, whereas methane–oxygen mixtures are only flammable up to 61 per cent methane[3], corresponding to 3·1 times as much methane as in a stoichiometric mixture.

The methane–perchloric acid flames were stabilized on cylindrical 'Pyrex' burners of internal diameter of 1·13–25·4 mm surrounded by a 65 mm jacket tube so that the flames were burning in an atmosphere of argon. The apparatus was preheated to 210° C by nichrome heating wire in order to maintain the perchloric acid in the vapour phase. All the flames were at atmospheric pressure.

Burning velocities, temperatures and burnt gas compositions were measured for flames containing 2–18 times as much methane as is required for a stoichiometric mixture. No carbon formation, as revealed by luminous yellow streaks or zones, was found with any of these flames. Burnt gas concentrations for methane, hydrogen, carbon monoxide, carbon dioxide and water in molecules per 100 molecules of initial methane are presented as a function of the mixture ratio, λ, in Fig. 1 (the diluent

water is not included). λ is defined as the fuel/oxidizer ratio in the mixture divided by the fuel/oxidizer ratio in a stoichiometric mixture. The other major product was hydrogen chloride and minor products were methyl chloride, chlorine and C_2 hydrocarbons.

The large amount of methane in the burnt gas is remarkable. It is possible to calculate an effective mixture ratio, φ, where φ is the fuel reacted/oxidizer ratio in the mixture divided by the fuel/oxidizer ratio in a stoichiometric mixture. The value of φ so calculated is 2 for any value of $\lambda > 2$. In other words, for flames containing more than twice the methane required for a stoichiometric mixture, the additional methane does not react and acts solely as a diluent. The flame temperatures measured with 0·002 in. Pt/13 per cent Rh–Pt thermocouples were compared with theoretical equilibrium temperatures calculated by (*a*) assuming that all the methane reacts, and by (*b*) assuming that only two moles react (that is, $\lambda = 2$) and that the rest of the methane acts solely as a diluent. The experimental temperatures confirm that methane-rich perchloric acid flames are in fact $\lambda = 2$ flames diluted with methane.

Carbon formation would not be likely in a $\lambda = 2$ flame (O/C ratio 2·0) and it is suggested that it does not occur in the richer flames because the flame temperature is insufficient. Addition of oxygen to methane-rich perchloric acid flames raises the flame temperature and carbon formation ensues. This observation supports the idea that carbon formation is not observed in the methane-acid flames because the flame temperature is too low.

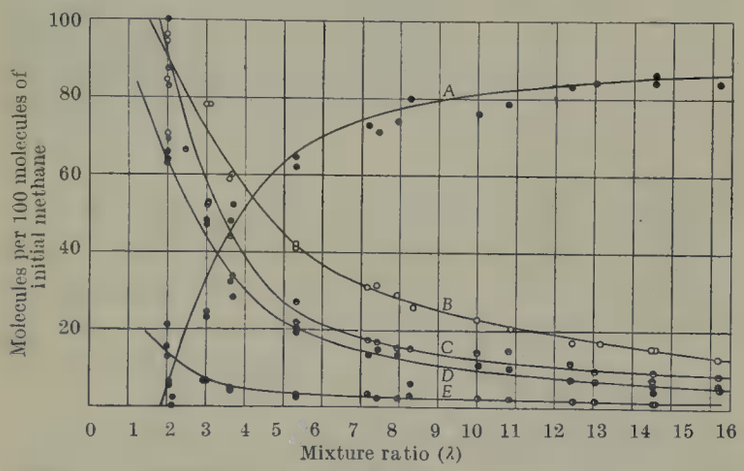

Fig. 1. *A*, Methane; *B*, water; *C*, hydrogen; *D*, carbon monoxide; *E*, carbon dioxide

The finding that only two molecules of methane react per molecule of perchloric acid is interesting. It cannot be directly compared to the situation with methane-rich oxygen flames since there is a paucity of sampling data. The only comprehensive study is that made by Townend in 1927 into the explosion of methane–oxygen mixtures at pressures of 6–150 atmospheres[4]. If it is assumed that the carbon produced in his experiments came from the pyrolysis of unreacted methane during the cooling of the post-flame gases, it is possible to calculate an effective mixture ratio, φ, in the same way as has been done for perchloric acid flames. For mixtures with $\lambda = 4$ and 5 the value of φ is close to 2. Further discussion is unwarranted since it is not certain that carbon is formed in this way. It would be interesting to sample premixed methane-rich oxygen flames under increased pressure to determine whether methane–oxygen flames are also essentially $\lambda = 2$ flames.

A mechanism can be proposed for the basic steps in a methane–perchloric flame. These must be different from those in a methane–oxygen flame since: (*a*) methane-perchloric acid flames have burning velocities about three times faster than the corresponding methane–oxygen flame[1]; (*b*) addition of oxygen to methane-rich perchloric acid flames results in the production of a second flame zone downstream of the acid zone[1]. Consequently, it is suggested that initial attack of the methane is by reaction with a chlorine–oxygen radical or a chlorine atom.

$$HClO_4 \rightarrow HO\cdot + ClO_3\cdot$$
$$ClO_3\cdot \rightarrow ClO\cdot + O_2$$
$$CH_4 + ClO\cdot \rightarrow CH_3\cdot + HOCl$$
$$HOCl \rightarrow HO\cdot + Cl\cdot$$
$$CH_4 + Cl\cdot \rightarrow CH_3\cdot + HCl$$

While ClO has been written as the chlorine–oxygen radical, since it has been suggested that it will be an efficient chain carrier[5], other chlorine–oxygen species could equally well be written in its place.

I thank Dr. A. R. Hall for his advice.

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BIOCHEMISTRY

Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons

Recognition of the molecular heterogeneity of enzymes in single organisms has offered a new dimension for studying the genetic control of enzyme synthesis. In the case of lactate dehydrogenase (LDH) five distinct types (isozymes) can be identified in most mammalian and avian tissues by the method of starch-gel electrophoresis[1–3]. Each isozyme is a tetramer formed by the association of two monomers, *A* and *B* (ref. 4), so that the polypeptide composition of LDH–1 through LDH–5 respectively may be written as follows: A_0B_4, A_1B_3, A_2B_2, A_3B_1 and A_4B_0. Genetic investigations[5–7] have shown that the synthesis of the *A* and *B* sub-units is under the control of two different structural genes, *a* and *b*. Hence the isozyme composition of each tissue depends on the relative activity of the genes at these loci.

The finding of unusual isozymes, 'band *X*' isozymes, in testes from sexually matured animals provided new information on the genetic control of LDH synthesis. The 'band *X*' isozymes are present only in mature testes, their appearance coincides with initiation of spermatogenesis, and they comprise the predominant isozymic forms of LDH in mature sperm[8–10]. These observations suggested that a third genetic locus was responsible for the synthesis of yet another distinct kind of LDH sub-unit, a *C*-polypeptide, in mature testis. Genetic evidence for the existence of this locus was the discovery of two allelic forms of the *c* gene in wild pigeons[11].

Therefore, at least three structural genes are involved in LDH synthesis. In the pigeon, assuming homozygosity for the *a* and *b* genes, three genotypes are possible: $\frac{abc}{abc}$, $\frac{abc'}{abc}$, and $\frac{abc'}{abc'}$. We now present evidence for allelism at the *b* locus, and thus have identified the genotypes that will be required for a future analysis of linkage relationships among the genes controlling LDH synthesis.

Racing homer pigeons, wild park pigeons, 'White Carneau', 'White King', and 'Silver King' pigeons were used in this investigation. Most of the racing homer and pure-bred pigeons were males, whereas the wild population comprised approximately equal numbers of males and females. Methods for preparation and electrophoresis of tissue homogenates and haemolysates, localization of

Table 1. Distribution of Classes I, II, and III in Three Different Pigeon Populations

Population	No. of pigeons in each class I	II	III	Allele frequencies L^B	$L^{B'}$
Wild	Obs. 864	56	1	0·97	0·03
	Exp. 863	57	1		
Racing homer 'White King'	207	0	0	1·0	0
'White Carneau' 'Silver King'	215	0	0	1·0	0

LDH isozymes in starch gel, dissociation and recombination of LDH polypeptide sub-units, and determination of total LDH activity were similar to those previously described[10].

A survey of the isozyme composition of tissues from approximately 1,000 wild pigeons showed that in terms of its isozyme phenotype each bird could be placed into one of three major classes which arbitrarily have been designated I, II and III. Examples of each class of pattern for breast muscle are shown in Fig. 1. The class I pattern exhibits five areas of enzyme activity, LDH-5, -4 and -3 being the darkest bands. The staining of LDH-1, -2 and -3 in the class II pattern of breast muscle is markedly diminished. Only three areas of activity are present in the class III pattern. The darkest band has the same electrophoretic mobility as LDH-5, and the other two bands migrate more slowly than LDH-4 and LDH-3.

Heart zymograms of the same pigeons from which the breast muscle patterns were obtained are also presented in Fig. 1. The class I pattern exhibits most of its activity

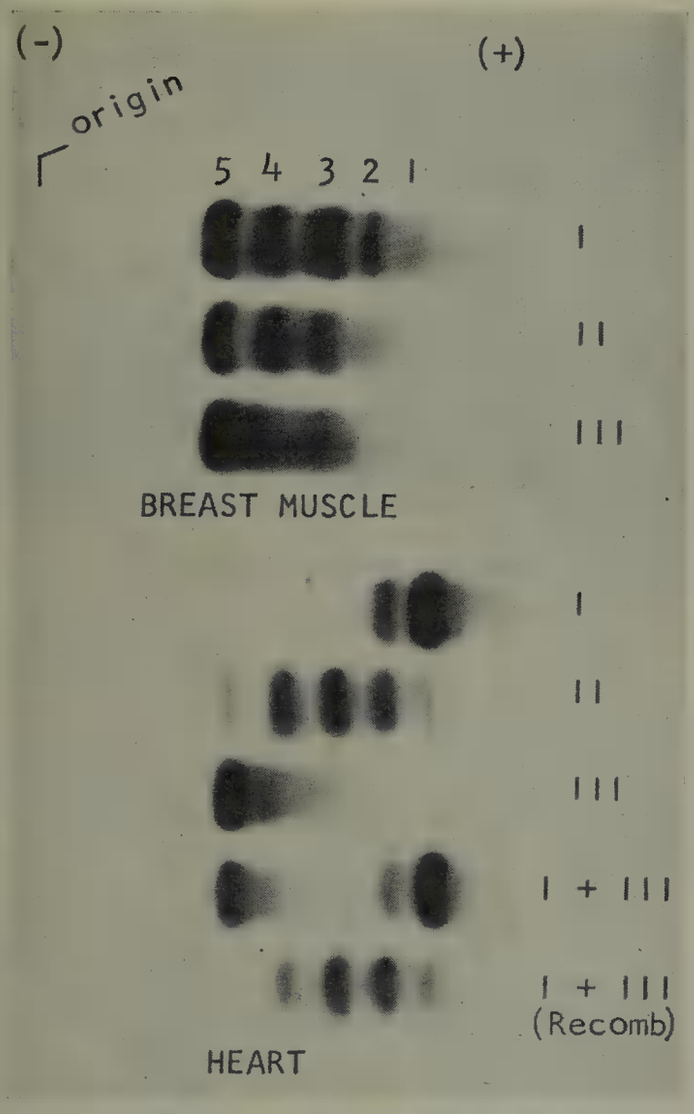

Fig. 1. Lactate dehydrogenase isozymes in breast muscle and heart from classes I, II and III pigeons. The homogenates were electrophoresed simultaneously in the same starch gel, and the conditions for identifying LDH were identical. The patterns of a mixture of equal parts of class I and class III hearts before and after treatment with sodium chloride and phosphate and freezing are designated I + III and I + III (Recomb) respectively

in the LDH-1 area, and less in LDH-2. Two minor bands can be seen on the anodal side of LDH-1. Five isozymes are present in the class II pattern, and the distribution of activity among the isozymes appears to be in the proportions of 1 : 4 : 6 : 4 : 1. The pattern of the class III heart, except for intensity of isozyme staining, is identical to that of class III breast muscle. Several other tissues from each pigeon were examined. In general, the differences in patterns of tissues with predominantly *B* polypeptides, for example, gizzard and kidney, paralleled the changes exhibited by heart. The characteristics of zymograms for tissues having mostly *A* polypeptides or equal amounts of each polypeptide were similar to those described for breast muscle.

In most mammalian and avian species adult heart muscle is rich in LDH-1 and LDH-2. Thus the presence of a major isozyme in the class III heart with an electrophoretic mobility identical to that of LDH-5 led to an investigation of some of its other physicochemical properties. When tissue homogenates from pigeons with class I patterns were heated at 70° C for 15 min, isozymes with two or more *A* polypeptides, that is, LDH-3, -4 and -5, were inactivated. Inactivation of the *B* polypeptides did not occur until homogenates were heated at 80° C for 15 min. The heat inactivation properties of the isozymes in homogenates of the class II and III heart were identical to those observed for the class I hearts. Inhibition investigations with *para*-amino-salicylic acid showed that concentrations of inhibitor which decreased LDH-5 activity in class I tissues did not alter the activity of the isozymes in homogenates of class I, II or III hearts.

These experimental results suggested that the major isozyme of the class III heart does not contain *A* polypeptides, but that it represents polymerization of a *B* variant, *B′*, the electrophoretic mobility of which is identical to that of the *A* polypeptide. Likewise the class II heart pattern can be explained by assuming that equal amounts of the normal and variant *B* polypeptides are being synthesized. If the polypeptide compositions of the major isozymes in the class I and III heart patterns are designated B_4 and B'_4 respectively, then the isozymic complement of class II heart would be as follows: $B_4B'_0$, $B_3B'_1$, $B_2B'_2$, $B_1B'_3$ and $B_0B'_4$. In order to determine if the *B* and *B′* polypeptides could combine to form functional enzyme units, a mixture of equal amounts of class I and III heart homogenates was treated with 0·5 M sodium chloride and 0·1 M phosphate and frozen for 24 h prior to electrophoresis[12]. As shown in Fig. 1, dissociation and re-association of the polypeptides resulted in the appearance of two new isozymes, and the pattern resembled the zymogram of a class II heart.

The nature of the two minor bands on the anodal side of the major isozyme in class III tissues is unknown. Since the electrophoretic mobilities of the *A* and *B′* polypeptides are identical, then only one isozyme should be visible in the class III patterns. Two minor bands are also present on the anodal side of LDH-1 in the pattern of class I heart. Perhaps these minor components are related in some way to the synthesis of the *B* and *B′* polypeptides.

Evidence for the genetic control of the three classes of isozyme patterns was obtained by measuring their relative frequency in different populations of pigeons. The data listed in Table 1 show that the distribution of the three classes in the wild-type population agrees with that expected according to the Hardy–Weinberg law for a single pair of alleles. All the racing homer and pure-bred pigeons exhibited class I patterns.

A conclusive demonstration of the mode of inheritance of the different class patterns was accomplished by carrying out appropriate breeding experiments. Adult wild pigeons were classified by electrophoresing haemolysates of blood obtained from a wing vein, and only class II pigeons were selected for the breeding colony. Twenty-

one offspring have been obtained from 11 matings, and the ratio of phenotypes (class I—*BB*: class II—*BB′*: class III—*B′B′*) is 4 : 11 : 6.

These findings unequivocally show that there is allelism at the *b* locus in wild pigeons. The biological significance of the three classes of patterns is unknown. The *B′* polypeptide has never been observed in tissues from racing homer or pure-bred pigeons, and only one of the 921 trapped wild pigeons was *B′B′*. This pigeon appeared healthy, and the total LDH activities of heart, thigh muscle, breast muscle and testis were normal. The successful production of *B′B′* pigeons in the breeding colony will facilitate more extensive studies on the selective advantage or disadvantage of the *B′* variant.

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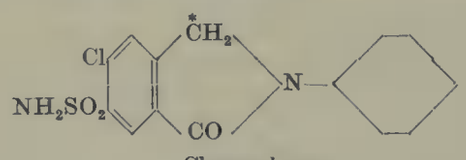
Metabolism of 5-Chloro-2-*cyclo*hexyl-1-oxo-6-sulphamoyl Isoindoline, Clorexolone (M and B 8430)

Clorexolone (M and B 8430) is a powerful oral diuretic[1] with pronounced antihypertensive properties even when administered in sub-diuretic doses[2]. It has certain structural features in common with chlorothiazide and with chlorthalidone, but is unique in containing the *cyclo*hexane-isoindoline structure.



Clorexolone

Although the metabolism in animals of *cyclo*hexane and its simple derivatives, for example, methyl*cyclo*hexanes, hexanols and hexanones, has been studied in detail by Williams *et al.*[3–5], there is little information about more complex derivatives. We have, therefore, examined the fate of clorexolone in experimental animals, and to a lesser degree in man, with two objects in mind. We wished, first, to establish that the compound was not stored in the body, and, secondly, we required information about the principal metabolic pathways operating in man and in the most closely related—in this biochemical context—animal species.

Preliminary investigations showed that colorimetric and spectrophotometric methods of estimating clorexolone and its congeners were insufficiently sensitive to permit quantitative experiments to be carried out with doses that approached those used therapeutically. They were, however, adequate to permit the conclusion that, in contradistinction to the thiazide diuretics, insignificant amounts of the parent compound were to be found unchanged in, for example, rat urine. We were also able to demonstrate, by thin-layer chromatography and infrared investigations, that the principal metabolite appeared to be monohydroxylated, possibly in the *cyclo*hexane nucleus. It was clearly undesirable, however, to rely solely on techniques which depended on excessive dosage —absorption, excretion and metabolic patterns may be atypical in such circumstances—and most of our preliminary work was therefore carried out with clorexolone labelled with carbon-14 in the isoindoline methylene group (see formula).

It will be seen from the tabulated results of the three experiments, in rats, one beagle dog and one whippet, that there is no significant accumulation of carbon-14 after periods ranging from 48 to 72 h.

Recovery of ^{14}C-labelled clorexolone after administration to two Wistar rats. Two Wistar rats, each weighing 220 g, received an oral dose of ^{14}C-labelled clorexolone (0·45 mg/kg) which was six times the dose necessary to produce a three-fold increase in sodium excretion. Each animal received 0·38 μc. of carbon-14

Table 1. Percentage Recovery of ^{14}C-labelled Drug

Sample	Time (h) 0–24	24–48	48–72	0–72
Urine	40·1	5·5	0·2	45·8
Faeces	31·0	18·2	0·0	49·2
Total	71·1	23·7	0·2	95·0

Recovery of ^{14}C-labelled clorexolone after administration to a beagle dog. A beagle weighing 12·5 kg received an oral dose of ^{14}C-labelled clorexolone (0·34 mg/kg), which was approximately three times the dose necessary to produce a three-fold increase in sodium excretion. The total amount of radioactive compound administered was equivalent to 5·0 μc. of carbon-14.

Table 2. Percentage Recovery of ^{14}C-labelled Drug

Sample	Time (h) 0–18	18–24	24–42	42–72	0–72
Urine	58·5	5·0	0·0	0·0	63·5
Faeces	—*	—*	27·5	0·2	27·7
Total	58·5	5·0	27·5	0·2	91·2

* The animal failed to produce any faeces in the first 24 h.

Recovery of ^{14}C-labelled clorexolone after administration to a whippet. A whippet weighing 5·7 kg received an oral dose of ^{14}C-labelled clorexolone (0·83 mg/kg), which was approximately eight times the dose necessary to produce a three-fold increase in sodium excretion. The total amount of radioactive compound administered was equivalent to 12·7 μc. of carbon-14.

Table 3. Percentage Recovery of ^{14}C-labelled Drug

Sample	Time (h) 0–24	24–48	48–72	0–72
Urine	95·4	3·2	0·0	98·6
Faeces	—*	4·0	2·2	6·2
Expired CO_2	0·0	0·0	0·0	0·0
Total	95·4	7·2	2·2	104·8

* The animal failed to produce any faeces in the first 24 h.

In the experiments involving the rats and the beagle, the carbon-14 content of the samples was determined by means of an end-window Geiger counter, whereas in the experiment with the whippet a liquid scintillation technique was used.

We wished to confirm that the main metabolic changes produced in the molecule involved the introduction of a single hydroxyl group and that the pattern of metabolism in the dog conformed closely to that operating in man. Preliminary investigations with the urine of volunteers had suggested that there was a great similarity between metabolic routes in man and dog.

Early experiments in which dogs received very large doses of non-radioactive clorexolone suggested the presence of three metabolites in the urine. After separation on silica-gel chromatoplates, using acetone as the developing solvent, these three compounds were visible as fluorescent spots when viewed in ultra-violet light of

257 mμ. Examination of the urine from the whippet which had received the radioactive compound, in a dose approximating to the therapeutic dose, confirmed that the whole of the administered carbon-14 was excreted in the form of these three metabolites, which will hereafter be referred to as compounds A_1, A_2 and B. These experiments also established that there were no significant differences in the metabolic patterns of large or small doses.

Having established this fact, it was then possible to administer daily doses of unlabelled clorexolone at 1 g/kg to a dog for 3 weeks so that enough urine could be collected to permit the isolation of these metabolites. Urine was collected daily and the three compounds were separated by extraction with ethyl acetate, thin-layer chromatography on silica and fractional crystallization.

Direct comparison, by chromatography and by infra-red and mass spectrometry, of metabolite A_2 with an authentic specimen of 5-chloro-2-(3′-hydroxy-*cyclo*hexyl)-1-oxo-6-sulphamoyl isoindoline showed that the two compounds were identical. Complete identification of the major metabolite, B, has not yet been established. Infra-red and nuclear magnetic resonance examinations suggest that it contains an equatorial hydroxyl group. The position of this group will be restricted to the 2′ or 4′ position, since A_2 is the equatorial isomer of the 3′ position, and the 1′ position has been eliminated by the nuclear magnetic resonance investigations. The chromatographic and infra-red characteristics of metabolite A_1 suggest that it also is monohydroxylated in the *cyclo*hexyl nucleus.

Three metabolites which had chromatographic and infra-red characteristics identical with those obtained from dog urine have been similarly isolated from human urine. Thirty litres of urine were collected over a 2-day period from a patient who was receiving 50 mg of clorexolone orally each day. Metabolite B was obtained crystalline, but the other two compounds were more difficult to purify and their identification depended on physical characteristics in solution.

It is evident that neither clorexolone nor its metabolites are stored in body tissues and that, although the compound itself is not excreted unchanged, metabolism and elimination occur solely and completely by way of oxidative monohydroxylations of the *cyclo*hexane nucleus.

We thank Miss H. N. Grant for preparing the ^{14}C-labelled material, and Drs. R. I. Reed and H. C. Hill for the mass spectrometric examinations.

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Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast

During re-investigation of an enzyme system from yeast previously reported to form 'activated' peptides[1] extensive proteolysis was noted and enzyme-catalysed hydroxylaminolysis of protein was suspected as the source of hydroxamate formation[2]. Acyl-enzyme complexes were visualized as intermediates rather than some form of 'activated' peptide originally suggested by Cooper *et al.*[1]. The main evidence for the latter hypothesis had been the appearance, after paper electrophoresis of the products of the reaction in the absence of hydroxylamine, of a band which gave a positive reaction to the hydroxylamine ferric chloride test. This test, however, may be misleading when conducted on paper[3]. The formation of hydroxamates from protein substrates by other proteolytic enzymes was also demonstrated[2] and is presumably analogous to the well-known transamidation reactions recorded by many authors[4–8]. Such reactions have only been unequivocally demonstrated using simple substrates but have been invoked to explain the formation of plasteins[9,10] and energy-independent incorporation of amino-acids into mitochondrial protein[11].

Some further characteristics of the yeast enzyme system are reported here. The crude yeast enzyme was prepared in essentially the manner described by Cooper *et al.*[1] and further purified by absorption on to a hydroxyapatite–cellulose column[12] in 0·01 M phosphate buffer, *p*H 6·8, and subsequent elution with 0·5 M buffer of the same *p*H. After extensive dialysis the protein was freeze dried. Enzyme reactions were carried out at 37° in 0·035 M *tris* buffer, *p*H 7·5, in the presence of 'low salt' hydroxylamine[13] (2·5–4·0 μmole) in a final volume of 3·3 ml. Additions of amino-acids, etc., were made in the same final volume after the respective solutions had been adjusted to *p*H 7·5. Reactions were terminated by the addition of 21·3 per cent (w/v) trichloroacetic acid in 0·43 N hydrochloric acid (2 ml.). After centrifugation the extinction of the supernatant at 280 mμ was observed. The supernatant (3·0 ml.) was also mixed with 6·6 per cent $FeCl_3.6H_2O$ in 0·1 N hydrochloric acid (0·5 ml.) and the extinction at 540 mμ observed after 5 min. Owing to the extreme sensitivity of the colour yield to minor changes in *p*H (ref. 14), the *p*H of the final mixture was observed and, before interpolating the hydroxamate concentration, the colour yield was corrected to *p*H 1·0, using graph of colour yield expressed as percentage of that at *p*H 1·0 plotted against *p*H. Glycine hydroxamate was used as a standard. Each determination was also corrected using a blank of identical composition to which trichloroacetic acid was added prior to addition of enzyme.

The formation of hydroxamates closely paralleled proteolysis in autolytic experiments (Fig. 1) and when additional protein substrate was present. Both activities are heat labile and reversibly inhibited by *p*-chloromercuribenzoic acid, thus resembling the more stable of the two enzymes obtained from yeast by Lenney[15]. Contrary to the earlier report[1], no evidence was found for any energy-dependent hydroxamate formation except

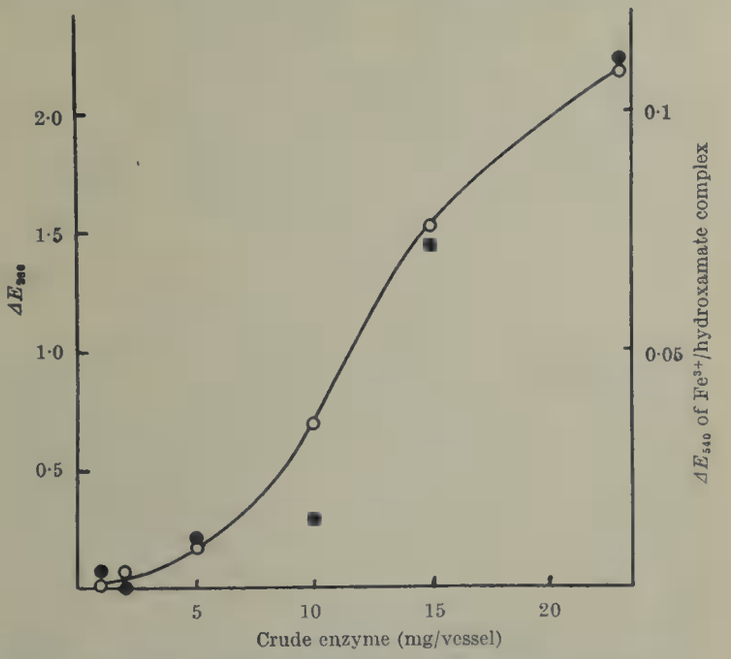

Fig. 1. Hydroxamate formation during autolysis of crude yeast enzyme in the presence of 2·7 mmole NH_2OH. Incubation time: 18 h. Increase in $E_{280\ m\mu}$ (○); increase in $E_{540\ m\mu}$ of Fe^{3+}/hydroxamate complex (●)

Table 1. Effect of ATP on Hydroxamate Formation during Autolysis of Yeast Enzymes

	Hydroxamate formed in 75 min (μmole)	
Crude enzyme (15 mg), NH_2OH (3·7 mmole)	1·55	1·75
,, ,, plus ATP (50 μmole), $MgCl_2$ (33 μmole)	1·24	1·43
,, ,, plus ATP, $MgCl_2$ and amino-acid mixture*	1·15	1·24
,, ,, plus amino-acid mixture only	1·00	0·85

* 8·3 μmole glutamic acid and tyrosine and 20·6 μmole methionine, phenylalanine and tryptophan in *tris* buffer, *p*H 7·5.

in the presence of amino-acids (Table 1). The highest yield of hydroxamate was, however, invariably observed in the absence of any additions. It is of interest to note that, in comparable experiments (Table 1), this system can produce up to 60 per cent of the hydroxamate observed in an apparently ATP-dependent system which was obtained by Schuurs *et al.*[16] by a somewhat different procedure, from baker's yeast. The Dutch authors recognized that their system was proteolytic in nature and, most significantly, that added peptides could not be activated[17].

It appears to be particularly difficult to interpret results based on the formation of hydroxamic acids when using crude enzyme systems which may contain proteases. This is especially true if trying to demonstrate peptide activation. Proof of such activation apparently requires a defined substrate and unequivocal identification of the reaction products[18].

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Hydrolysis of Two 17α-Oestradiol Glycosides by Rabbit Tissue Glycosidases

It has recently been shown[1,2] that the rabbit excretes 17α-oestradiol as the 3-glucuronoside-17α-*N*-acetylglucosaminide. To the best of our knowledge this is the first *N*-acetylglucosaminide of a non-sugar to be found in animals, although β-*N*-acetylglucosaminidase is widely distributed in animal tissues[3]. It was the object of the work recorded here to determine whether the steroid *N*-acetylglucosaminide was effectively hydrolysed by the tissue enzyme in rabbits, and whether the enzyme distribution, when measured with this naturally occurring substrate, was the same as that found by previous investigators[3,4] who used synthetic substrates. We also wished to compare the relative ability of different tissues to remove the glucuronic acid and the *N*-acetylglucosamine from the oestradiol molecule.

Four rabbits were injected subcutaneously with 50 mg/day of oestrone benzoate in sesame oil. On the second day an intravenous injection of 50 μc. of 17β-oestradiol-6,7-^{3}H (sp. act. 150 μc./μg) was also given. Oestradiol-3-glucuronoside-17α-*N*-acetylglucosaminide was isolated from the urine as previously described[2]. A sample of the di-glycoside was incubated in 0·5 M acetate buffer at *p*H 5·0 with β-glucuronidase ('Ketodase', Warner-Chilcott). Oestradiol-17α-*N*-acetylglucosaminide was extracted from the buffer with ethyl acetate and purified by countercurrent distribution[1]. After crystallization from aqueous methanol, the product had a specific activity of 156 μc./mmole. Another sample of the di-glycoside was incubated in 0·5 M citrate buffer at *p*H 4·2 with almond emulsin in the presence of 10 mg/ml. of saccharo-1,4-lactone. The buffer was extracted exhaustively with ethyl acetate and the extract distributed through 99 countercurrent transfers in ethyl acetate–butanol–water, 3 : 1 : 4. A single radioactive product with a *K* value of 5·2 was located. This material was dissolved in water and, on the addition of a few drops of chloroform, yielded a semi-crystalline product of specific activity 167 μc./mmole. The infra-red spectrum of this compound confirmed its structure as an oestrogen glucuronoside, and treatment of the material with β-glucuronidase yielded 17α-oestradiol.

Oestradiol-17α-*N*-acetylglucosaminide has an extremely low partition coefficient between chloroform and water[1]. That of the 3-glucuronoside is even lower. A convenient method of measuring the hydrolysis of these glycosides by tissue enzymes was therefore to extract the released steroid from the incubation medium and assay the radioactivity in the extract by liquid scintillation spectrometry. In our hands this method has given accurate and reproducible results, and chromatography of the extracts has shown that the chloroform-soluble radioactive material liberated from both glycosides by tissue enzymes is unchanged 17α-oestradiol.

Organs were dissected from rabbits immediately after killing. Aliquots of the tissue were homogenized in acetate buffer at *p*H 4·5 and in citrate buffer at *p*H 4·2. A 10 per cent w/v ratio of tissue to buffer was used in each case. Aliquots of homogenate were diluted to 10 ml. with the appropriate buffer, and the radioactive glycosides (about 100 μg/g tissue) were added in 50λ of ethanol. β-Glucuronidase activity was assayed in acetate buffer using oestradiol-3-glucuronoside as substrate, and β-*N*-acetylglucosaminidase was assayed using oestradiol-17α-*N*-acetylglucosaminide in citrate buffer[5].

At buffer concentrations of 0·5 M, the β-glucuronidase of both liver and kidney displayed the two *p*H optima, at 4·5 and 5·2, observed by previous workers[6], with that at *p*H 4·5 predominant. If lower buffer concentrations were used, effects such as those obtained by Fishman[7] were observed, in that several optima appeared, at rather variable, but usually quite acid, *p*H values. A similar problem was encountered when attempts were made to assay β-*N*-acetylglucosaminidase at low buffer concentrations, and a smooth *p*H curve with an optimum at 4·2–4·4 was only obtained at a citrate concentration of 0·5 M. Accordingly, the assays of both enzymes were carried out in 0·5 M buffers.

A comparison of the ability of rabbit tissues to hydrolyse the steroid glucuronoside and the *N*-acetylglucosaminide is shown in Table 1. The overall distribution of both enzymes is broadly similar to that reported by previous workers[3,4] for the rat and mouse. Female rabbit liver shows a much higher glucuronidase activity toward the steroid substrate than any other tissue. The activity is nearly three times as high as that of male liver. In the rabbit, as in other species[3], the epididymis has a very high level of β-*N*-acetylglucosaminidase, and this tissue has the lowest ratio of β-glucuronidase/β-*N*-acetylglucosaminidase activity. The spleen of both sexes is remarkably active in hydrolysing both oestradiol glycosides—the female spleen

Table 1. Hydrolysis of 17α-Oestradiol Glycosides by Rabbit Tissues *in vitro*

Results are expressed as μg of substrate hydrolysed per g of tissue at 37°. Incubations with liver tissue were for 4 h; with all other tissues the incubation time was 21 h

Tissue (M, male; F, female)	Oestradiol-17α-*N*-acetyl-glucosaminide	17α-Oestradiol glucuronide	Ratio of β-glucuronidase to β-*N*-acetylglucos-aminidase activity
Liver M	4·64	248·0	53·4
Liver F	6·52	696·0	106·7
Kidney M	38·64	51·87	1·34
Kidney F	48·93	69·72	1·42
Spleen M	128·73	378·0	2·94
Spleen F	191·10	372·96	1·95
Plasma M	2·94	7·14	2·43
Plasma F	2·73	9·24	3·38
Large intestine F	38·01	67·20	1·77
Small intestine F	30·45	90·72	2·98
Uterus	14·28	25·83	1·81
Ovary	16·59	54·81	3·30
Epididymis	157·29	55·86	0·36
Testis	13·86	45·57	3·29

having the highest β-*N*-acetylglucosaminidase activity, on a wet weight basis, of any tissue tested.

The results indicate that oestradiol-17α-*N*-acetyl-glucosaminide is cleaved, under *in vitro* conditions, by the *N*-acetylglucosaminidase present in rabbit tissues. Whether this reaction has any significance *in vivo* is being investigated.

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Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin

Using a fly bioassay technique, Kunze and Laug[1] reported the presence of toxicant in liver, kidney, abdominal fat and urine of rats fed dieldrin or aldrin. They observed that the toxicant in kidney and urine occurred mainly in males, with traces only in females. This toxicant in male kidney and urine differed from either dieldrin or aldrin because it could be readily destroyed by treatment with 2 per cent alcoholic KOH, a property not shared by the parent substances. Since these observations, a number of investigators[2–6] have reported findings which indicate the presence of derivations of dieldrin and aldrin in tissues and urine. These are assumed to be metabolites and can be most readily distinguished from dieldrin and aldrin by their increased hydrophilic properties.

It is of interest to investigate these metabolic derivatives further by the use of electron-capture gas chromatography, a tool of great sensitivity in the detection and isolation of chlorinated insecticides.

Mature male and female Sprague–Dawley rats within a weight range of 250–350 g were fed diets containing dieldrin or aldrin for 120 days. Two levels of each insecticide were fed, namely 1 p.p.m. and 25 p.p.m. These corresponded respectively to average daily intakes of 0·015 mg and 0·375 mg per rat. A control group was maintained on a standard 'Purina' laboratory chow. Analyses showed this diet to contain no detectable aldrin or dieldrin within the limits of detection in a 100-g sample, but traces of DDT and related derivatives were found.

During the experiment, urine samples were collected at intervals and at termination a final sample together with liver, kidney and abdominal fat tissues. All specimens were extracted with ethyl ether, the extracts made to volume and suitable aliquots prepared for gas chromatography. Careful consideration was given to the possibility that the 25 p.p.m. levels could be grossly toxic, or sufficiently embarrassing to the metabolic machinery to cause qualitative differences in response between high and low dosages.

The gas chromatographic analyses were carried out in a Barber-Colman 'Model 10' gas chromatograph, utilizing a 4-ft. U-shaped glass column. The column consisted of a 2·5 per cent coating of *SF*-96 on 'gas Chrom *P*.', 100–120 mesh. The temperatures of the column and the electron-capture detector were 170° and 200° C, respectively. The detector cell applied voltage was set at 18 V for optimum detection of chlorinated hydrocarbons. The system was able to detect readily 15×10^{-6} μg of aldrin, 20×10^{-6} μg of isodrin and 40×10^{-6} μg of dieldrin or endrin.

Illustrative tracings shown in Fig. 1 are representative of gas chromatographic determinations made on urines

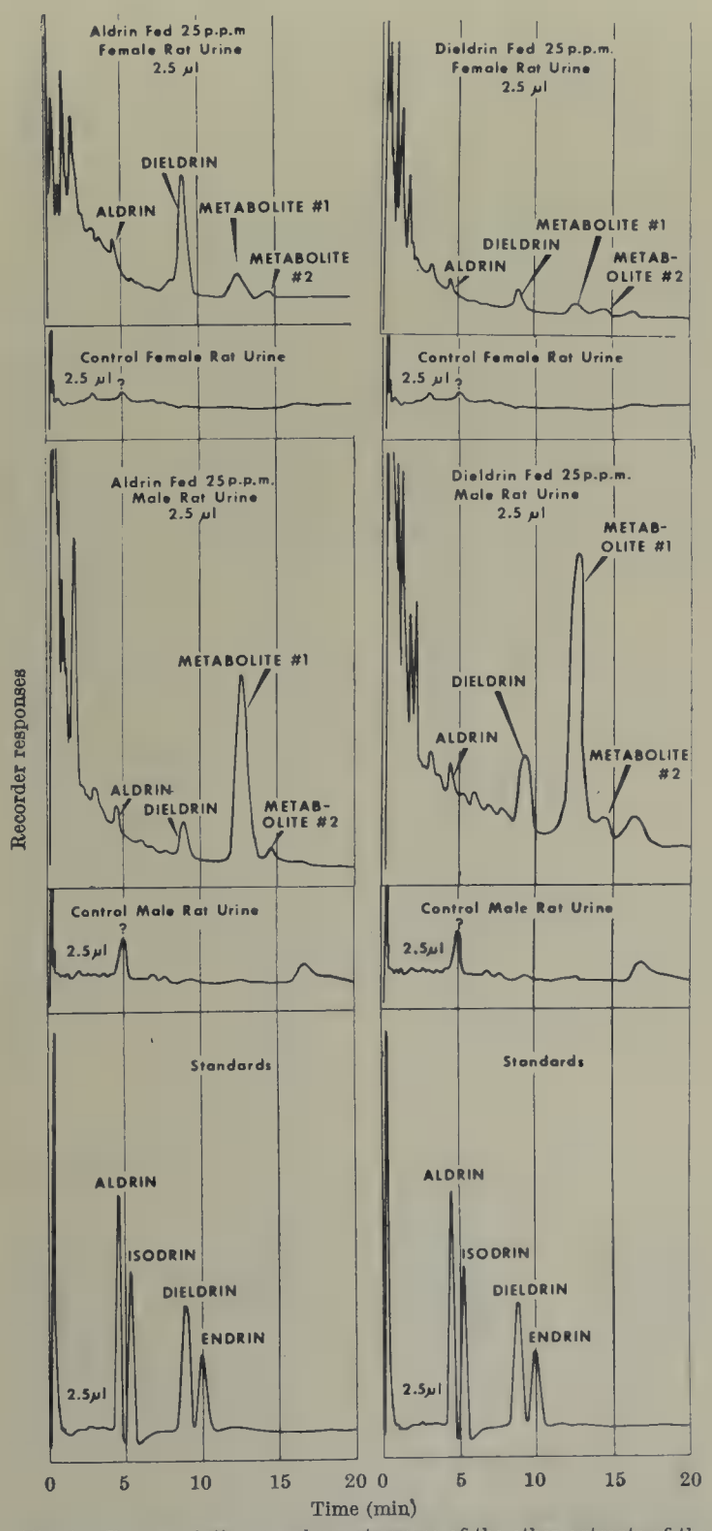

Fig. 1. Representative gas chromatograms of the ether extracts of the urine from control and experimental rats. In all cases 2·5 μl. solution injected represented 15 μl. urine. Standard solution: 2·5 μl. represents $2{\cdot}25 \times 10^{-4}$ μg aldrin, $2{\cdot}5 \times 10^{-4}$ μg isodrin, $2{\cdot}25 \times 10^{-4}$ μg dieldrin, 3×10^{-4} μg endrin, respectively

from animals exposed to 25 p.p.m. levels of dieldrin or aldrin. It can be seen that relatively large quantities (approximately 5:1) of a metabolite designated No. 1, having a retention time longer than any of the drin standards, appears predominantly in male urine, and only small amounts in female urine. Smaller quantities of a second metabolite No. 2, with even longer retention time, can be observed in about equal quantities in male and female urine. The latter peaks are not to be confused with two of unknown origin that appear in the control urines of both sexes. It should be especially noted that aldrin and dieldrin produce the same metabolites No. 1 and No. 2. From the type of column used, the longer retention times for both metabolites indicate that they are more polar than the parent substances. Furthermore, on hydrolysis of the urine extracts with alcoholic KOH, both metabolites disappear. This is in contrast to dieldrin and aldrin, which are alkali-stable. The epoxidation of aldrin to dieldrin both *in vitro* and *in vivo* has been well established[7] and can be readily confirmed in our tracings. What is of interest, however, is that the animals which were fed dieldrin also appear to excrete small amounts of aldrin in the urine. These results cannot be explained on the basis of aldrin contamination of dieldrin, within the range of detectability of aldrin stated earlier. Preliminary results, notably on kidney extracts, show that the profile of metabolites in this tissue is similar to that in urine, with preponderance of metabolite No. 1 in the male kidney and sensitivity to alkaline hydrolysis.

No gross toxic effects at the 25 p.p.m. levels of dieldrin and aldrin were observed. Further, the gas chromatographic tracings made on extracts from the urine of animals exposed to 1 p.p.m. did not differ from those exposed to the higher levels except quantitatively. Shorter oral exposure to aldrin or dieldrin of rats indicates a similar profile of metabolites. It is of interest to note, however, that in some preliminary intravenous trials only minimal amounts of metabolites were observed in urine.

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PHYSIOLOGY

Electrical Activity in Human Uterine Muscle Strips

In spite of increasing interest in functional disorders of the human uterus and clinical investigation, the obstetrician has little control apart from stimulation of uterine contractions. Premature labour leading to prematurity is at the present time the greatest single preventable cause of neonatal morbidity and mortality[1-3]. Much of the present-day investigation attempts empirically to use smooth muscle relaxants to prevent premature activity, without a basic understanding of the mechanism of normal uterine muscle control. It is not known, for example, how oxytocin exerts its stimulatory action on the gravid uterus.

We have succeeded in recording action potentials from human uterine muscle strips. The amplitude and speed of propagation of the potentials have been correlated with the mechanical action in non-pregnant and pregnant human myometrial strips.

In the non-pregnant patients the muscle strips were carefully cut from the anterior fundal wall immediately following hysterectomy. Care was taken to keep the serosal surface intact. The strip was trimmed to approximately 45 × 5 × 2 mm and immediately placed in a solution of Krebs solution, previously saturated with 95 per cent O_2 and 5 per cent CO_2. Time elapsing from removal to muscle bath was approximately 5 min. In the pregnant cases the strips were taken from the incision site, which was made in the low transverse cervical region. Strips were occasionally removed from the anterior fundal region during sterilization procedures 2–4 days post-partum.

The muscle was then tied, serosal side up, to two clamps. One clamp was fixed while the other was connected to a Grass strain gauge. The muscle was then immersed in the bath of Krebs solution, maintained at 37° C with 95 per cent O_2 and 5 per cent CO_2 bubbling. The strain gauge was connected to one channel of a Beckman–Offner multichannel recorder. Two blunt glass-pore electrodes were then separately placed on the surface of the muscle (on the intact serosal surface) a measured distance, 5–10 mm, apart. Each electrode was then connected to separate channels of the Beckman–Offner recorder. The level of the bath solution was adjusted to cover the muscle surface by 5–10 mm to prevent drying and to maintain adequate nutrition and temperature control (Figs. 1 and 2).

In the pregnant strips, the initial action potentials are recorded immediately before the onset of the contraction (Fig. 3). They have a characteristic configuration: an initial positive deflexion followed by a rapid negative deflexion, a rapid return to base-line with a slight overshoot to the positive side, then a slow decay to base-line.

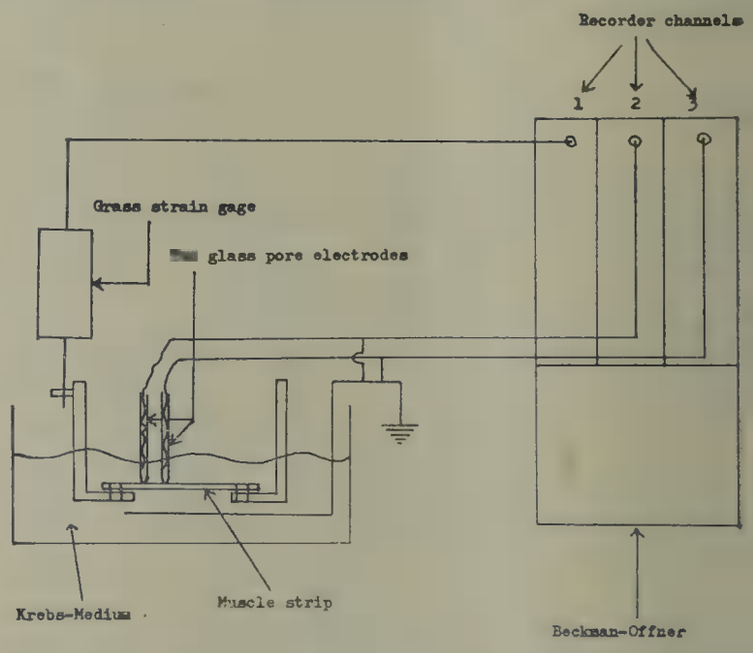

Fig. 1. Schematic illustration of technique used to record both mechanical activity and action potentials

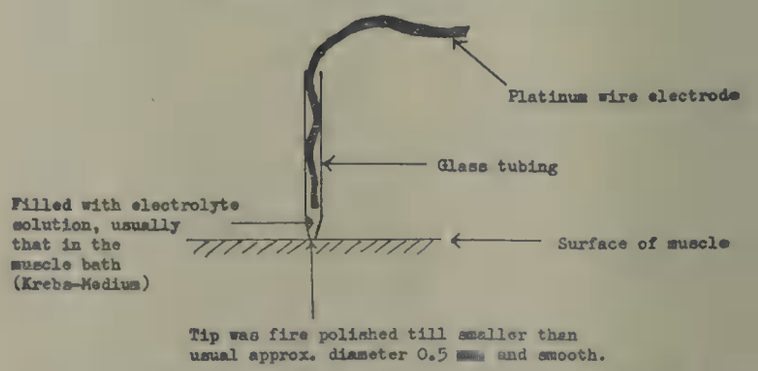
Platinum wire electrode
Glass tubing
Filled with electrolyte solution, usually that in the muscle bath (Krebs-Medium)
Surface of muscle
Tip was fire polished till smaller than usual approx. diameter 0.5 and smooth.

Fig. 2. Smooth glass-pore electrode used for recording action potentials

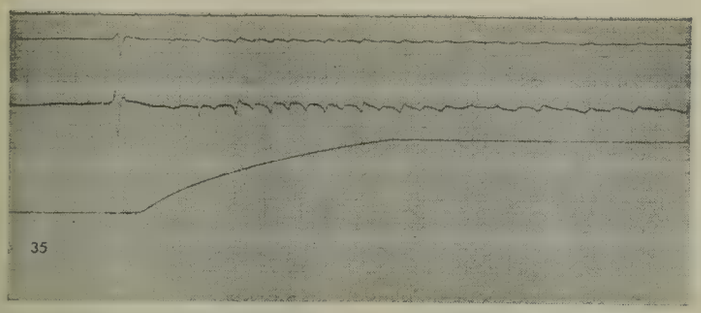

Fig. 3. Electrical and mechanical activity recorded from a pregnant, human uterine muscle strip. Compare the interval between the initial action potentials to that from the non-pregnant muscle. Paper speed 30 mm/sec or 6 squares/sec

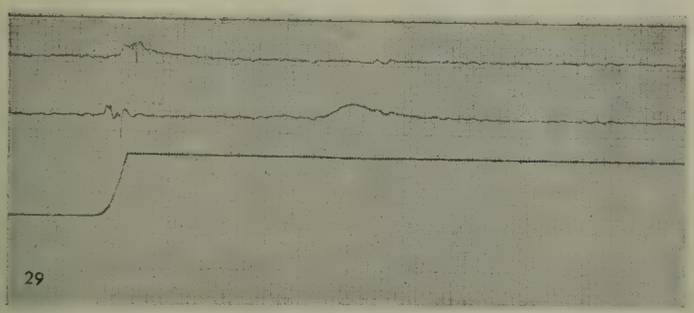

Fig. 4. Electrical and mechanical activity recorded from a non-pregnant human uterine muscle strip. Note the onset of the tension prior to the recording of the action potentials, also the distance separating action potentials from the two recording electrodes. Paper speed 15 mm/sec or 3 squares/sec

Subsequent potentials are of lesser amplitude and do not show the initial positive deflexion.

In the non-pregnant strips the initial potentials are recorded after the contraction has begun (Figs. 3 and 4). The initial action potentials from a representative non-pregnant muscle averaged 0·189 mV and were propagated at an average of 0·69 cm/sec. The comparable figures for the initial action potential from a typical pregnant muscle were 0·35 mV and 13·3 cm/sec. This wide difference between voltages and conduction velocities of action potentials has also been found to occur in non-pregnant and pregnant rabbit uterine strips[4].

If we assume that only an action potential can initiate uterine contraction, then in the non-pregnant muscle the recording of appreciable tension change before the first recorded action potential must result from the slow propagation of electrical activity. Calculations based on the maximum time required for propagation of the action potential from either end of the muscle to the electrode indicated that the slow conduction velocity could have explained the measured delay in the appearance of the action potential following the onset of contraction. Further studies will be required to establish that slow propagation is indeed the explanation for the delayed onset of action potentials in non-pregnant uterine strips. If the electrodes are randomly placed on the muscle surface and action potentials originate at random spots, we would expect on occasion to record the action potentials before, or earlier in, the contraction. To date there has been no evidence to suggest a specialized pacemaker in the uterus, though pregnant strips always propagated action potentials from the fundal area distally in the instances when the spatial origins of the strip were known.

The action potentials, following the initial one which were recorded throughout the contraction in the pregnant strips, appeared to be isolated and non-propagated. The absence of the initial positive deflexion also suggested that they are not produced by a wave of depolarization from an adjacent area. It is tempting to assume that the difference in the electrical activity in pregnant and non-pregnant muscle is due to the great difference in hormone balance present, and work is in progress on this aspect of the problem. This work was supported by the Canadian Medical Research Council (grant *MA* 1656).

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Release of Plasminogen Activator by the Isolated Perfused Dog Kidney

Blood contains a proenzyme, plasminogen (profibrinolysin), which is converted to the proteolytic enzyme plasmin (fibrinolysin) by plasminogen activator. The level of plasminogen activator in the blood is increased in conditions of stress and after injection of epinephrine or nicotinic acid[1,2]. The origin of blood plasminogen activator has not been established with certainty although it has been suggested that it may be derived from the plasminogen activator activity found in association with the endothelial lining of vessel walls[3–5]. In dogs, blood fibrinolytic activity can be increased by intravenous injection of a number of vasoactive drugs[6,7], but vasodilative drugs (for example, histamine) appear to be more effective than vasoconstrictive ones[8].

We have studied the effect of histamine on the release of plasminogen activator by the isolated perfused dog kidney. A total of seven experiments were performed, using healthy mongrel dogs anaesthetized with 'Nembutal' (25 mg/kg). One kidney was removed to serve as control. The contralateral kidney of the dog was carefully freed from surrounding tissues, and the renal artery and the renal vein were ligated at their junction with the aorta and vena cava, respectively. A polyethylene catheter was inserted into the renal artery and another into the renal vein and the kidney perfused *in situ* with unoxygenated Locke's solution (*p*H 7·3) at 37° C until the effluent fluid had become completely clear and virtually free of red blood cells. Clearance of blood required from 180 to 480 ml. of Locke's solution. The effluent fluid during this initial perfusion period was collected in 100-ml. flasks. The kidney was then further perfused with Locke's solution and the perfusate collected as serial 5-ml. aliquots. At intervals histamine phosphate (Burroughs Wellcome and Co., Tuckahoe, N.Y.) was injected through the arterial catheter. The histamine was dissolved in Locke's solution at a final concentration of 1 mg base per ml. Each dose was 100 μg of histamine base per kg body-weight of the intact dog. The rate of perfusion was maintained at about 33 ml. per min throughout the whole experiment. Occasional red blood cells in samples of perfusate were removed by centrifugation at 4° C. The fibrinolytic activity of the perfusate was determined on unheated[9] and heated[10] bovine fibrin plates prepared as described previously[11]. The protein content of the perfusate was measured by reading the optical density at 280 mμ and was expressed in terms of μg tyrosine per ml. Glutamic oxalacetic transaminase levels were measured according to the method recommended by the Sigma Chemical Co. (St. Louis, Missouri) and using their reagents.

For the histological study of plasminogen activator in kidney tissue, a modified Todd's technique[12] was used. Fibrin slides were prepared by clotting 0·6 ml. of a mixture of equal volumes of bovine fibrinogen solution (Bovine Fibrinogen, Behringwerke, Marburg/Lahn, Germany), 2 g per cent, and calcium chloride solution (0·05 M), with 0·1

ml. thrombin solution (Bovine Thrombin Topical, Parke, Davis and Co., Detroit, Michigan), 20 N.I.H. units per ml., on a 5 cm × 2 cm area of a standard microscopic glass slide. Veronal–hydrochloric acid buffer according to Owren[13] was used to dissolve fibrinogen and thrombin. A block of tissue including a pyramid and its overlying cortex was excised in a transverse plane from the centre of the kidney. It was frozen at −20° C and sections 10μ thick were cut with a freezing microtome. These sections were placed on the fibrin slides and incubated in a moist chamber at 37° C. After different intervals, slides were removed from the incubator and fixed in formaldehyde vapour and 10 per cent formol–saline solution. Afterwards, the slides were stained with Harris's haematoxylin and lithium carbonate and mounted in glycerine jelly.

The perfusate obtained before injection of histamine had low fibrinolytic activity and sometimes had no activity at all. In each experiment the injection of a first dose of histamine through the arterial catheter was followed immediately by the appearance of increased amounts of plasminogen activator in the perfusate. The perfusate had no measurable lytic activity on heated bovine fibrin plates, indicating that the active material was plasminogen activator. Injection of histamine was not followed by a significant change in the level of glutamic oxalacetic transaminase or in the protein content of the perfusate. Intra-arterial injection of a second dose of histamine after a short interval had no effect on the release of plasminogen activator into the perfusate. The results of a typical kidney perfusion experiment are given in Fig. 1.

The plasminogen activator content of frozen sections of the perfused, histamine-treated kidney and the contralateral control kidney was compared in each experiment; in 4 of the 7 experiments the histamine-treated kidney showed considerable decrease of the plasminogen activator activity normally found in association with the vasa recta[14] of the subcortical zone. Fig. 2 demonstrates the decreased amount of plasminogen activator activity in a perfused kidney as compared with the normal, contralateral kidney of the same dog.

The presented data provide evidence that, in an isolated perfused organ, histamine stimulates the release of plasminogen activator into the perfusing fluid. The considerable depletion of plasminogen activator in the subcortical zone of four perfused kidneys suggests that the wall of the vasa recta is a source of releasable plasminogen activator. The fact that the level of glutamic oxalacetic transaminase, a common intracellular enzyme, does not increase suggests that plasminogen activator release is not accompanied by a significant degree of cellular necrosis.

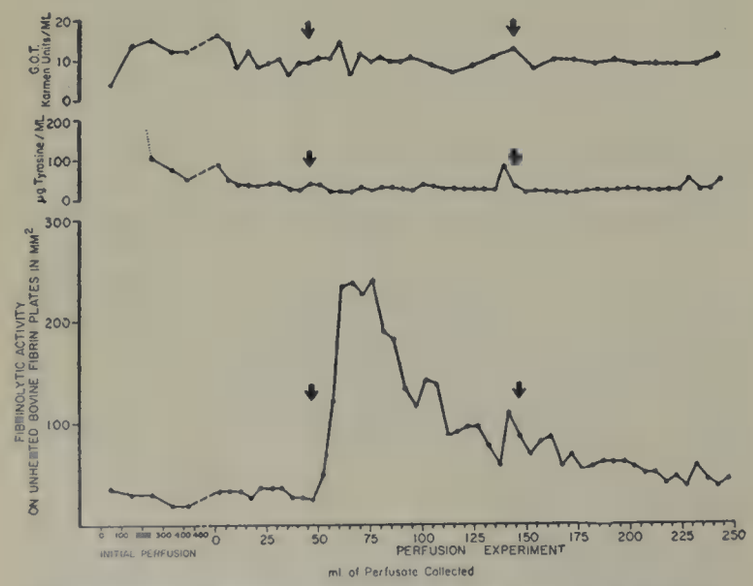
G.O.T. Karmen Units/ML
μg Tyrosine / ML
FIBRINOLYTIC ACTIVITY ON UNHEATED BOVINE FIBRIN PLATES IN MM²
INITIAL PERFUSION
PERFUSION EXPERIMENT
ml of Perfusate Collected

Fig. 1. Fibrinolytic activity, glutamic oxalacetic transaminase level and protein content of perfusate of a kidney perfusion experiment. Histamine, 100 μg per kg body-weight of the intact dog, was injected through the arterial catheter at the times indicated by the arrows

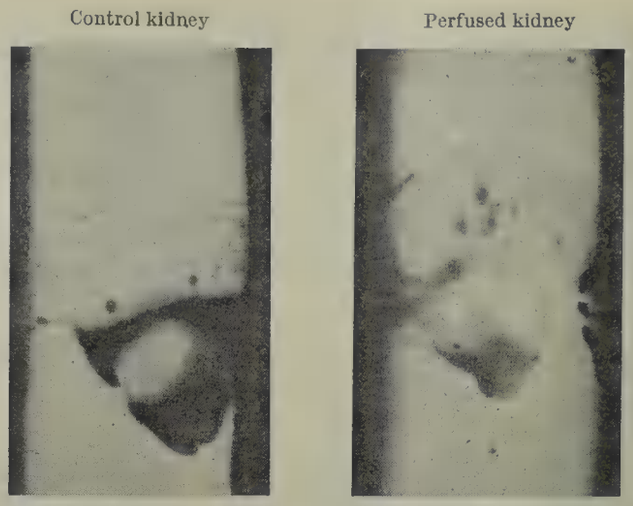

Fig. 2. Unstained sections of a control and perfused, histamine-treated kidney of a dog. Both sections were incubated at 37° C on unheated fibrin slides for 130 min. The dark areas indicate lysis of the fibrin layer. It can be seen that there is complete lysis over the subcortical zone in the control kidney whereas the section of the perfused kidney shows little lytic activity

Tissular hypoxaemia may have played a part in the histamine-induced release of plasminogen activator since these experiments were performed on anoxaemic organs. It cannot be ruled out that histamine releases plasminogen activator by a direct effect on endothelial cells of small vessels or capillaries, although it has been impossible to demonstrate such an effect on walls of large veins. Injection of histamine into an isolated venous segment of the dog or incubation of an everted jugular vein of the dog in histamine solution does not decrease plasminogen activator content of the vessel wall[8,15].

A more likely explanation is that the release of plasminogen activator by histamine is attributable to the vasoactive effects of this drug. Assuming that the amount of plasminogen activator released is directly proportional to the area of perfused inner blood vessel surface, the opening up of a large number of small blood vessels by histamine in the perfused organ can be expected to increase plasminogen activator release.

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Thinning of the Human Cornea on Awakening

Much remains unknown about the mechanism whereby the cornea maintains its shape, thickness, and transparency. Maurice[1] points out that the observed negative water pressure inside the cornea and the positive

pressure in the aqueous humor and tears bathing the cornea should lead to continuous swelling and, therefore, to corneal cloudiness. Some process, yet unknown, maintains the *in vivo* cornea at constant thickness and transparency. Mishima and Maurice[2] showed that whatever process operates to maintain constant corneal thickness, the process can be disturbed by closing the eyes for an hour or more. They showed that the rabbit cornea thinned by four per cent over a period of two hours after the eye was opened. The thinning was attributed to evaporation of solvent (water) from the tears to produce a hypertonic tear film which withdrew water from the cornea by an osmotic effect. Conversely, when the eye was closed and thus bathed in isotonic tears, the cornea swelled. They considered the other two environmental factors which change when the eye is closed, namely temperature and oxygen tension, to be ineffective in changing thickness. The corneal temperature of the closed eye is about 4° C above that of the open eye. The oxygen tension of the epithelial surface of the closed eye must be about 50 mm mercury (the average between venous and arterial blood) whereas the open eye in air is exposed to 155 mm mercury oxygen tension.

The slow blinking rate of the rabbit (once every 10 min, compared with the human rate of 12 blinks per min) suggests that evaporation from the human eye may not be the same as from the rabbit eye. We have, therefore, measured the thinning of the *in vivo* human cornea after the eye is opened on awakening[3].

The subject, a Caucasian female 20 years of age, was instructed to tape her left eye closed immediately on awakening in the morning, usually after 6–8 h of sleep. The tape was removed in the laboratory about 30 min later and corneal thickness measured as a function of time for several hours. Normal blinking was allowed at all times. Corneal thickness measurements were made by using a Vickers optical beam splitter fitted into one tube of a binocular microscope on a Thorpe-type slit-lamp[4]. The slit-lamp projects a vertical light beam about 1 mm wide and 1 cm long. In traversing the cornea it illuminates, by scattered light, the cross-section of the cornea that is observed in the microscope and beam splitter. By adjusting the beam splitter to give first a coincident image and then side-by-side images, the thickness can be obtained through a calibration which uses five corneal contact lenses of known thickness and refractive index. The calibration curve is shown in the insert of Fig. 1. The theory of such *in vivo* corneal thickness measurements has been reviewed by von Bahr[5].

Because of the large spacial variation in the thickness of the human cornea, a critical feature of this experiment was repeating the measurements at a constant corneal position. All parts of the slit-lamp assembly were rigidly fixed by clamps. The subject was held in a reproducible position by a dental-mould mouthpiece and head-bar which were fixed to a sturdy mounting on a weighted table. The standard fixation light on the slit-lamp was covered with a disk about 3 cm in diameter with a 1-mm hole in the centre. This provided a small fixation light to reduce eye movements. Only when the biomicroscope was properly aligned was a bright image produced by specular reflexion from the endothelium, and tear surface.

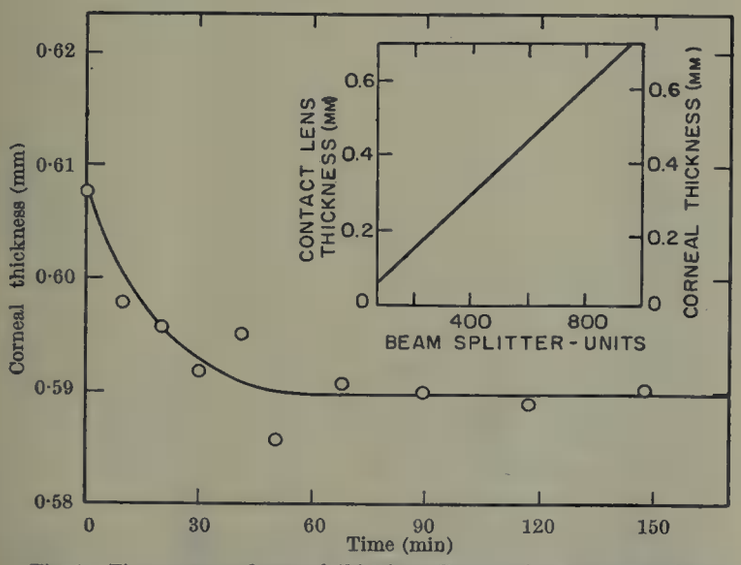

Fig. 1. Time course of corneal thinning after opening eye on awakening from 6 h sleep. Insert shows calibration curve of beam splitter with contact lenses of various thicknesses. Corneal thickness is 0·92 times contact lense thickness at a given beam splitter reading for the optical system used here

Measurements were made on four separate days. About ten settings of the beam splitter were made in each 5-min period for the first 80 min and then in 5-min periods separated by 15-min intervals for the remaining 80 min.

Fig. 1 shows a corneal thickness as a function of time. The cornea on awakening appears to be about 3·6 per cent thicker than normal. The normal-thickness base line was obtained by continuing the measurements, in some cases, to 6 h. The thickened cornea returns to normal in about 1 h.

The maximum corneal swelling of about 4 per cent is the same for rabbit and man. However, the rabbit cornea returns to normal thickness in 0·5 h (ref. 2) whereas the human cornea requires about 1 h. This can be explained by the lower blink rate of the rabbit. At this lower rate the tears concentrated by evaporation are replaced only infrequently, whereas in man there is frequent replacement of tears and, therefore, less-concentrated tears. Corneal thinning is probably caused by extraction of water by the osmotic pressure of hypertonic tears. A lower hypertonicity, accompanying the higher blinking rate in man, would give slower thinning.

We thank Miss Mary Linda Morrison for being the subject. The Vickers beam splitter was loaned to us by the Corneal Center, New York. This work was supported in part by U.S. Public Health Service research grants *NB* 04810 to R.B.M. and *HE* 06796 to I.F.

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[3] Mishima, S., *Arch. Ophthal.*, **73**, 233 (1965), has reported thinning of the human cornea on opening the eye (Fig. 7*B*) but did not give his experimental method nor the conditions under which the measurements were made. Our results are in good agreement with Mishima's.
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Use of Polyethylene Glycol in Investigations of Absorption of Fat

In 1957 Borgström *et al.*[1] introduced the use of unabsorbable polyethylene glycol (PEG) for quantitative investigations of absorption of fat, protein and carbohydrate from the intestine. Stimulated by their communication, a number of authors[2–4] used PEG to examine absorption. (After the administration of PEG and a food mixture of known composition in the withdrawn specimen of the intestinal contents the PEG concentration and investigated nutrients are estimated. From the two values the percentage ratio of the absorbed nutrient is calculated.)

Wiggins and Dawson[5], however, directed attention to the fact that in the stomach a dissociation of PEG and the fat from the food mixture takes place; PEG remains mainly in the aqueous phase, which passes more rapidly into the intestine than the lipid phase. In a preliminary

Table 1. Influence of PEG on the Lipolytic Activity of Human Duodenal Juice

Final conc. of PEG/ml.	Lipolytic activity as percentage of activity of control specimen: Juice 1 (2·5 per cent l.)	Juice 2 (3·5 per cent l.)	Juice 3 (11 per cent l.)*
0·5	100	88·5	104
1·0	68	100	104
2·5	—	76·5	90
4·0	76·5	85·5	100
5·0	50·0	76·5	101

* Percentage of lipolysis in control specimens after incubation for 30 min. Composition of incubation medium: 0·2 ml. olive oil; 0·2 ml. phosphate buffer *p*H 6·5; 0·5 ml. PEG solution (1, 2, 5, 8, 10 mg/1 ml. duodenal juice).

experiment we investigated the stability of the food mixture (1 part olive oil, 4 parts dried milk, 1 part dextrose, 8 parts water) during incubation with human gastric juice, 1 : 1, at 37° C. It was revealed that the presence of PEG in a concentration of 5 mg/ml. distinctly promotes the breakdown of the food mixture.

The adverse effect of PEG on the stability of the emulsion system of the mixture brings up the question of how this substance participates in the breakdown of fats by pancreatic lipase. So far as we know, reports on this problem are lacking; we investigated therefore the effect of PEG on lipolysis in a model experiment *in vitro*.

As a source of lipase we used an aqueous 0·1 per cent extract of acetone powder of hog pancreas and fresh human duodenal juice. Olive oil served as substrate. The final concentration of PEG (molecular weight, 4,000) varied between 0·5 and 5 mg/ml. (In the experiment three different preparations of PEG were used. We thank Prof. B. Borgström and Prof. A. M. Dawson for kindly supplying specimens of PEG.) The exact composition of the incubation medium is given in the legend of Table 1 and Fig. 1. The degree of lipolysis was assessed from the difference of free fatty acids (NEFA) estimated in the incubation medium by Dole's method[6] at the onset of and after the incubation. The results are expressed as the percentage activity of the control specimen which did not contain PEG.

In the experiment with pancreas extract it was found that in the presence of PEG the lipolytic activity was inhibited, depending on the concentration of PEG. As is apparent from Fig. 1, the inhibition is in general the greater, the smaller the amount of lipase in the incubation medium.

In the subsequent experiment we replaced the synthetic digestive mixture by human duodenal juice. It was revealed that also in this system PEG exerts an inhibitory effect (Table 1). Contrary to the first experiment, the effect of PEG rather varied; it attained a decrease up to 50 per cent, however, not observed with low lipase activity. The inhibitory effect of PEG may be explained perhaps by its adverse action on the stability of the food mixture. This assumption requires, however, further confirmation. In any event, our finding supports the view[5] that PEG is not very suitable for the accurate quantitative investigation of absorption of fat. A satisfactory reference substance should have the following properties: (*a*) it must not be absorbed; (*b*) it must not influence gastric emptying; (*c*) it must be soluble in the same medium as the substrate; (*d*) it must not affect the digestion and absorption of the investigated substance.

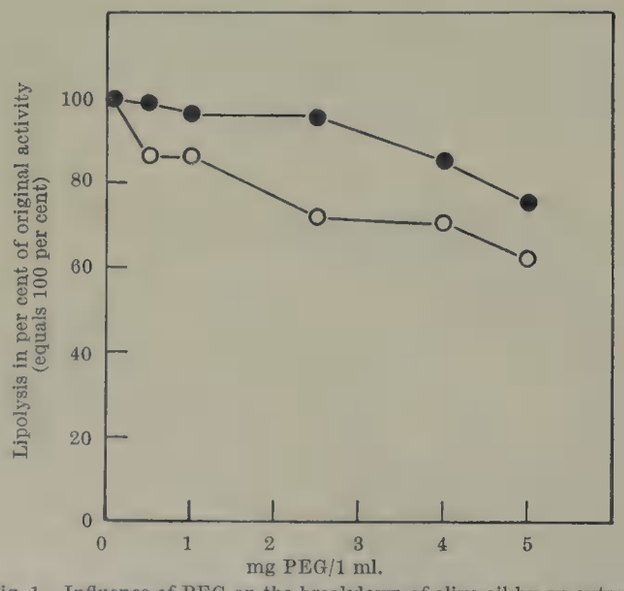

Fig. 1. Influence of PEG on the breakdown of olive oil by an extract of hog pancreas. Composition of incubation medium: 0·1 ml. olive oil; 0·1 ml. 0·2 M $CaCl_2$; 0·2 ml. *tris* 0·2 M, *p*H 8·0; 0·2 ml. lipase extract; 0·25 ml. PEG solution 2, 4, 10, 16, 20 mg/1 ml.; 0·1 ml. H_2O. Incubation period 30 min. *x* axis, final concentration of PEG; *y* axis, percentage lipolytic activity of control specimen (without addition of PEG). ●, 0·1 per cent lipase extract; ○, 0·02 per cent lipase extract

From experiments of Wiggins and Dawson as well as ours presented in this communication, it is obvious that PEG does not meet the latter two conditions, so far as lipids are concerned.

Experiments *in vitro* revealed that PEG inhibits lipase from hog pancreas and human duodenal juice; it is therefore not very suitable for use as a reference substance for investigating absorption of fat, at least in conditions with low lipase activity.

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PHARMACOLOGY

Some Effects of Drugs on the Isolated Superfused Carotid Body

The isolated superfused carotid body preparation described by Eyzaguirre and Lewin[1], and used by them to investigate some properties of the carotid body, is a useful method for investigating chemoreceptor activity without need for concern about changes in the blood supply. This communication reports the results of an examination of the effects of a number of compounds on the activity of chemoreceptors in such a preparation. Some of these drugs would normally be expected to modify the blood flow through the carotid body and hence indirectly to affect the chemoreceptor activity. The changes in activity which they induce thus, here, represent the direct actions of drugs on the chemoreceptors. In addition, the effect of changes in the calcium ion concentration of the superfusate has been investigated because there is evidence[2–4] that transmitter release at a number of sites may be related to calcium ion concentration.

The experiments were performed on the carotid body and sinus nerve removed from 18 cats lightly anaesthetized with sodium pentobarbitone. The superfusion technique was similar to that described by Eyzaguirre and Lewin[1] and the bathing fluid was Krebs's solution equilibrated with 95 per cent oxygen, 5 per cent carbon dioxide, maintaining the *p*H at 7·4. This was also confirmed by direct measurement. Five reservoirs were available so that different drug mixtures could be applied.

The sinus nerve was laid on a stainless steel back plate and dissected under liquid paraffin floating on the Krebs's solution in which the carotid body was suspended. Action potentials were recorded through a bipolar platinum wire electrode and the nerve impulses could be photographed from an oscilloscope and counted on a ratemeter with a chart recorder giving a permanent record.

The effects of a number of drugs were tested on the chemoreceptor activity when the rate of flow of the superfusate was increased so that the discharge fell to a minimum, and when a maximum hypoxic response was produced by raising the carotid body into the liquid paraffin.

The former could be held constant over long periods and the latter was repeatable.

Acetylcholine (ACh) in a concentration of 10^{-4}–10^{-5} g/ml. always caused slight excitation, though adrenaline (A), noradrenaline (NA) and isopropylnoradrenaline (IP) excited in concentrations of 10^{-5} g/ml. Lower concentrations were without effect. In two experiments, hexamethonium bromide (C6) 10^{-5} g/ml. blocked the ACh excitation and was without effect on the hypoxic response, in one the converse was the case and in the other this concentration was without effect. C6 alone did not change the resting rate.

The most consistent blocking effects were seen with nethalide which, in concentrations of 10^{-5} g/ml., invariably depressed the resting discharge and depressed the hypoxic response. In addition, it blocked ACh and IP excitation. The results with dichloro-isopropylnoradrenaline (DCI) were more variable, but either it had no effect or depressed the resting rate and hypoxic response at 10^{-5} g/ml.

Phenoxybenzamine in concentrations of 10^{-5} g/ml. increased the discharge rate at maximum flow, an effect which was slow in onset and long-lasting. In one test it blocked the excitation previously produced by NA. Piperidyl-methyl benzodioxane (933*F*) in low doses, 10^{-6}–10^{-5} g/ml., similarly caused excitation. Neither drug had constant effects on the maximum hypoxic discharge rate, which was little changed.

Bretylium tosylate at a concentration of 10^{-5} g/ml. depressed the resting discharge and maximum hypoxic frequency and blocked ACh excitation. Its administration was also usually associated with depression of action potential amplitude.

The effect of reserpine could not be adequately tested, for it usually precipitated out in the superfusate.

In four experiments the effect of changing the calcium ion concentration was investigated. Superfusion with Krebs's solution containing no calcium chloride caused an increase in the spontaneous activity at maximum flow rate, while raising the calcium concentration by two or four times depressed this activity.

The experiments have also provided the opportunity for examining the statistical properties of the nerve impulse pattern. In three experiments single unit chemoreceptor afferent preparations were made and the distribution of intervals between action potentials was analysed in the manner described by Biscoe and Taylor[5]. This analysis showed that the mean interval and the standard deviation of twenty successive intervals were similar for mean intervals larger than approximately 300 msec, but that the *S.D.* became progressively smaller than the mean for intervals shorter than this. This result is similar to that previously obtained with the *in vivo* perfused carotid body[5]. An equality of mean and standard deviation is a property of a random interval distribution[6].

It seems clear that ACh, NA, A and IP can all cause excitation of carotid body chemoreceptor afferents, presumably in this preparation by an effect other than on the vascular bed. High concentrations are required, and it is not necessary to suppose that ACh, for example, is acting here as a chemical transmitter, for its excitant effects on nerve endings are well known[7,8]. The results with hexamethonium are equivocal, and on the basis of this evidence there can be no serious disagreement with Douglas[9], or with the criticisms of the notion that ACh is a transmitter at this site[10,11]. One of the more interesting results is the effect of nethalide, a β-adrenergic blocking drug, for its effect was consistently obtainable and unlike bretylium, for example, it did not depress the action potential height, an effect which may be related to the local anaesthetic action of bretylium[12,13]. According to the theory expounded by Douglas and Rubin[2], and Douglas and Poisner[3], and the evidence of Philippu and Schümann[4], on release of catecholamine from the adrenal gland, the changes in activity produced by calcium should be the converse of those seen here if transmitter release were calcium dependent. But the changes in activity with the calcium ion concentration are explicable in terms of direct effects on nerve endings[14], and do not yield any information about transmitter release, if indeed a transmitter exists.

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Lethal Effects of Aggregation and Electric Shock in Mice treated with Cocaine

Sensory stimuli provided by aggregation[1] or submaximal electric shock[2] become incompatible with life in mice treated with low doses of amphetamine. Effects of aggregation[3,4] or electric shock[5] on brain levels of norepinephrine are also modified by amphetamine. Amphetamine inhibits the uptake of norepinephrine by tissues *in vitro*[6,7] and *in vivo*[8]. Termination of the biological actions of norepinephrine to a large extent depends on the tissue uptake or 'recapturing' of the free norepinephrine[9]. It is therefore postulated that toxicity of sensory stimuli in treated mice is partly due to the interference by amphetamine of the 'recapturing' of norepinephrine liberated from sympathetic nerves due to stimulation.

Cocaine, like amphetamine, also blocks the tissue uptake of norepinephrine *in vitro*[10,11] and *in vivo*[12,14]. The present report describes the lethal properties of sensory stimuli in the mice treated with cocaine.

Swiss albino random-bred male mice weighing 25–28 g were placed in shock apparatus or aggregation cages immediately after intramuscular injection of cocaine. All other drugs were injected intraperitoneally. The shock apparatus was similar to one described by Weiss *et al.*[2], while aggregation was according to Cohen and Lal[1]. In isolation, only one mouse was placed in each cage, while 10 mice in a cage constituted aggregation. Shocks were provided for 4 h. There were no deaths with chlorpromazine or reserpine when given alone and the mice were aggregated. Reserpine produced some deaths among shocked mice.

Data given in Table 1 suggest that aggregation or shock became lethal to mice treated with sub-lethal doses of cocaine. As is seen from Table 2, the enhanced lethality of aggregation was completely prevented by pretreatment with adequate doses of chlorpromazine or reserpine.

Table 1. Toxicity of Sensory Stimuli in Mice treated with Cocaine

Cocaine, mg/kg	Experimental conditions	4-h mortality Dead/injected	%
0*	Isolated	0/30	0
	Aggregated	0/30	0
	Shocked	0/20	0
50	Isolated	0/14	0
	Aggregated	1/20	5
	Shocked	5/24	20
100	Isolated	1/10	10
	Aggregated	13/20	65
	Shocked	15/24	63

* Mice were injected with 90 mg of sodium chloride per kg of body-weight.

Table 2. Effect of Chlorpromazine and Reserpine on Stimulated Mice treated with Cocaine*

Drug	Dose (mg/kg)	Experimental conditions	4-h mortality Dead/injected	%
Sodium chloride	90	Aggregated	13/20	65
Chlorpromazine	5	Aggregated	9/10	90
Chlorpromazine	20	Aggregated	0/10	0
Reserpine	5	Aggregated	1/10	10
Sodium chloride	90	Shocked	15/24	63
Chlorpromazine	5	Shocked	6/10	60
Chlorpromazine	20	Shocked	1/10	10
Reserpine	5	Shocked	4/10	40

* 100 mg of cocaine per kg of body-wt. was injected 1 h after chlorpromazine or 4 h after reserpine into all the mice.

Chlorpromazine also protected shocked mice. Effect of reserpine in shocked mice was inconclusive since in shocked mice reserpine exhibited some toxicity of its own.

Recently several investigations have demonstrated rapid and specific uptake of circulating norepinephrine by tissues[13,15,16], sympathetic nerve endings[17], and isolated granules from nerve endings[18]. In isolated perfused heart, more norepinephrine is lost by tissue uptake than by enzymatic metabolism[17]. Denervation[19], which depresses tissue uptake of norepinephrine, produces supersensitivity to injected norepinephrine.

On the basis of these observations it has been suggested that intensity or duration of biological action of circulating or locally released norepinephrine is limited to a large extent by 'recapturing mechanisms'. Interference with these mechanisms by drugs or other procedures may result in increased and prolonged pharmacological effects of exogenously or endogenously released norepinephrine. Present studies of effects of sensory stimuli in presence of cocaine or amphetamine provide *in vivo* evidence of an exaggerated sympathetic syndrome. Excessive sensory stimuli liberate norepinephrine through central and peripheral sympathetic discharge. Inactivation of liberated norepinephrine by 'recapturing mechanisms' is prevented by cocaine or amphetamine, resulting in prolonged and intense action of norepinephrine on tissue, causing tissue damage and death. Pretreatment with normal noradrenergic blocking agents may provide protection against the exaggerated sympathetic syndrome.

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Interference by the Carcinogenic 4-Nitroquinoline *N*-Oxide of Tryptophan and Indole Uptake in *Escherichia coli*

The potent water-soluble carcinogen 4-nitroquinoline *N*-oxide (NQO) is remarkably versatile and thus represents a good means of discerning pre-requisites for carcinogenesis. NQO condenses readily with sulphydryl groups; is mutagenic to bacteria, tobacco mosaic virus, and *Aspergillus niger*[1]; inhibits incorporation of phosphorus-32 into nucleic acids of Ehrlich ascites carcinoma cells[2]; induces nucleolar 'caps' in Chang liver cells[3]; and inhibits growth of the flagellates *Ochromonas danica*, *Euglena gracilis*, of *Corynebacterium bovis*, and of the photosynthetic purple bacterium *Rhodopseudomonas palustris*. These growth inhibitions are competitively annulled by L-tryptophan; D-tryptophan is also effective. The competitive annulment ratio is 0·2 mg/ml. tryptophan : NQO 2 μg/ml.

This growth competition suggests that NQO uptake is mediated by a tryptophan transport mechanism[4]. To investigate this possibility, two tryptophan-requiring mutant strains of *E. coli*, *T*3 and *T*24 (cultures obtained from Dr. C. Yanofsky), both utilizing tryptophan or indole were investigated. NQO was tested for interference with the inducible tryptophan permease of both strains by a modified Burrows and DeMoss[5] technique: 24 mg (dry weight) log-phase cells were collected on a 'Millipore' cellulose filter (0·05μ pore size) and washed for 80 sec with isotonic C- and N-free medium and the filtrate discarded. C- and N-free medium + tryptophan (or indole) at equimolar concentrations was then washed over the cells for 40 sec and this filtrate collected. Absorbancy of the medium + tryptophan or indole was measured before and after exposure to NQO by means of a Beckman *DU* spectrophotometer set at 280 mμ.

Table 1. Interference by 4-Nitroquinoline *N*-Oxide of L-Tryptophan and Indole Uptake in *E. coli*
(Readings as absorbance at 280 mμ)

Additions	Readings before exposure to cells	Control (after 40 sec washing)	Cells exposed to 4×10^{-4} M NQO for 5 min (readings taken after 40 sec washing with C- and N-free medium)
NQO 4×10^{-4} M	0·002	0·0019	0·003
L-Tryptophan $0·5 \times 10^{-4}$ M	0·43	0·30	0·42
Indole $0·5 \times 10^{-4}$ M	0·195	0·04	0·15

Introduction of NQO at 4×10^{-4} M (shown by viability investigations to be non-inhibitory in a culture of this density) into the growing culture completely inhibited uptake of L-tryptophan and inhibited indole uptake by 80 per cent within 5 min (Table 1). Both strains behaved alike.

These results are construed as supporting our earlier idea that ability to deceive tryptophan transport systems may underlie the carcinogenic specificity of polycyclic carcinogens.

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HAEMATOLOGY

Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human Platelets

The aggregation of washed human platelets *in vitro* by adenosine diphosphate (ADP) requires calcium and is stimulated by the addition of platelet-free plasma[1]. The identity of the plasma activity has been the subject of recent conflicting reports. Hellem and Owren[2] have suggested that the plasma factor is that entity missing in von Willebrand's disease; but others[3,4] have found that ADP-induced platelet aggregation proceeds normally in plasma from patients with established von Willebrand's disease. McLean *et al.*[5] and Cross[4] have indicated that fibrinogen may be the plasma co-factor, and Caen[6] has

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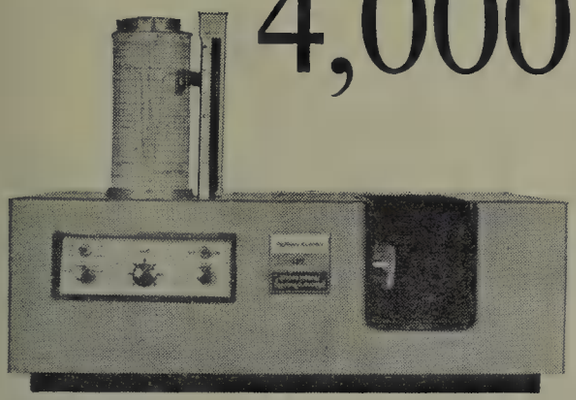

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recently suggested that both fibrinogen and the anti-von Willebrand factor participate in ADP-induced platelet aggregation.

The experiments described here were undertaken in an effort to clarify the nature of the stimulatory activity of plasma on ADP-induced aggregation of washed human platelets. Platelets were collected and processed with siliconized equipment from normal subjects and from a patient with established von Willebrand's disease, using the acid-citrate anticoagulant of Aster and Jandl[7]. Platelet-rich plasma was prepared by centrifugation at 275*g* for 15 min at 23° C. Platelet pellets were obtained by further centrifugation at 2,500*g* for 5 min at 4° C. The platelets were resuspended with a Pasteur pipette in Gaintner's platelet buffer[8], modified in that the final glucose concentration was 2 g/l. and the final *p*H was 6·5. The platelets were washed twice in the same buffer and were finally resuspended in the same medium at a final concentration of 7×10^8/ml. The suspended platelets could be kept in this medium at 4° C for as long as 4 h with no evidence of spontaneous aggregation and with no diminution of sensitivity to ADP. Platelet aggregation was measured by turbidometric methods similar to those of Born and Cross[1] and O'Brien[9]. To 1 ml. of platelet suspension were added saline, the plasma or serum fraction to be examined, and calcium chloride. The *p*H of the mixture was then titrated to 8·3 by the addition of 0·1 N sodium hydroxide, and the reaction was initiated by the addition of ADP. The final volume of the reaction mixture was 2·5 ml. The final calcium chloride concentration was $1{\cdot}7 \times 10^{-3}$ M. Final concentration of ADP was 10^{-4} M. The optical density of the suspension was determined at 600 mμ in a spectrophotometer placed over a rotating magnet. The platelets were continuously stirred by a 'Teflon'-coated bar placed in the cuvette. The optical density contributed by the platelets was determined by adjusting to zero the absorbance of an identical reaction mixture in which platelet buffer replaced the platelet suspension. The optical density of the platelets was determined immediately and at 60 sec following the addition of ADP. The decrease in absorbance was expressed as the percentage of initial optical density:

$$\frac{\Delta \text{ optical density in 60 sec}}{\text{initial optical density}} \times 100$$

The stimulation of ADP-induced platelet aggregation by saline, by citrated human plasma heated to 56° for 10 min (from which the precipitated fibrinogen had been removed by centrifugation), by thrombin-free human serum heated at 56° for 10 min, and by human fibrinogen (fraction I–0) is shown in Table 1. In the presence of saline alone some aggregation of platelets occurred. There was little additional aggregation when heated plasma or heated serum was added individually. Whole plasma produced a marked increment of platelet aggregation. Fibrinogen alone stimulated platelet aggregation, but not to the same extent as whole plasma. In contrast, the addition of heated plasma and fibrinogen together, or of heated serum and fibrinogen together, reconstituted the entire activity of the whole plasma. Identical results were obtained using whole plasma, heated plasma or serum drawn from the patient with von Willebrand's disease (Table 1). Heated plasma dialysed extensively against 0·154 M saline was as effective as non-dialysed plasma when added to fibrinogen. The entire activity of the heated plasma could be recovered in the fraction precipitated by ammonium sulphate at 45 per cent saturation. In other experiments purified human fibrinogen (94 per cent clottable protein) was used instead of fraction I–0 (70 per cent clottable protein). No significant differences were noted when the more purified preparation was used.

The concentration dependence of the ADP-stimulated reaction on whole plasma, on heated plasma in the presence of non-limiting amounts of fibrinogen, and on

Table 1. Influence of Whole Plasma, Heated Plasma, Heated Serum, and Fibrinogen on ADP-induced Aggregation of Washed Human Platelets*

Addition (ml.)	Δ Absorbance (per cent) Normal†	von Willebrand‡
Whole plasma, 0·8	29·1	36·5
Fibrinogen (5·5 mg/ml.), 0·3	8·9	8·9
Heated plasma, 0·8	2·9	4·1
Heated serum, 0·8	7·8	5·5
Heated plasma, 0·8 + fibrinogen, 0·3	27·9	37·0
Heated serum, 0·8 + fibrinogen, 0·3	29·9	32·5

* Stimulation of ADP-induced aggregation above that seen in the presence of saline alone. The average Δ absorbance of 5 experiments with saline alone was 9·3 per cent.

† Average of 4 experiments.

‡ In these experiments washed normal platelets were used. No significant differences were observed when von Willebrand platelets were used with either normal or von Willebrand plasma or serum.

fibrinogen in the presence of non-limiting amounts of heated plasma is shown in Figs. 1 and 2. The percentage decrease in optical density due to saline alone has been subtracted from each point plotted. The shape of the whole-plasma curve is complex. A plateau of activity was repeatedly observed between 0·05 and 0·12 ml. of plasma, suggesting the interaction of more than one plasma component. No plateau was evident in the heated plasma plus fibrinogen curve which demonstrated consistently greater activity than that of the corresponding concentration of whole plasma until maximum activity was reached. Similarly, no plateau was observed in the fibrinogen curve, which reached maximal activity at a final fibrinogen concentration of 0·34 mg/ml. Similar curves were obtained with the von Willebrand's plasma.

It is apparent that the heat-stable protein is not consumed in coagulation, since heated serum was as effective as heated plasma. It is also evident that the heat-stable plasma factor required for ADP-induced platelet aggregation is not missing in the plasma of a patient with classical von Willebrand's disease. The mechanism whereby fibrinogen stimulates the ADP-induced aggregation is not immediately apparent. It is clear, however, that the participation of fibrinogen is not dependent on a thrombin catalysed conversion to fibrin, since the aggregation proceeds fully in the presence of heparin. In addition, electron micrographs of ADP-induced platelet aggregates show no evidence of fibrin formation. These findings are similar to those reported by Caen[6].

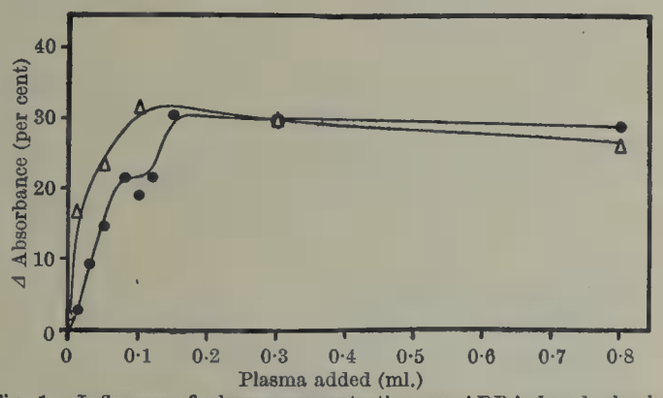

Fig. 1. Influence of plasma concentration on ADP-induced platelet aggregation. Solid circles indicate effect of increments of whole plasma. Open triangles indicate effect of increments of heated plasma in the presence of non-limiting fibrinogen concentration

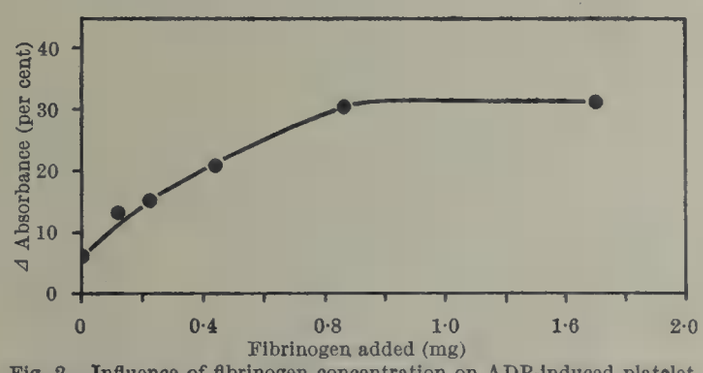

Fig. 2. Influence of fibrinogen concentration on ADP-induced platelet aggregation, in the presence of non-limiting heated plasma concentration

These experiments demonstrate that at least two plasma factors, fibrinogen and a heat-stable plasma protein, distinct from the anti-von Willebrand factor, participate in ADP-induced platelet aggregation in the presence of human plasma.

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A Modification System for Fingerprinting Haemoglobin

The technique of 'fingerprinting' developed by Ingram[1] has proved to be of great value in the molecular analysis of haemoglobins. In principle, it is a combined paper electrophoresis and chromatography of the peptides released by enzymatic digestion of haemoglobin. This enables the location of a probably occurring difference to a particular peptide. The method has proved its importance especially in the detection of amino-acid differences in normal and abnormal haemoglobins. This communication describes a modified system for fingerprinting haemoglobins.

In Ingram's procedure the first stage, paper electrophoresis, is performed in Michl's volatile buffer[2]: pyridine–glacial acetic acid–water (20 : 0·4 : 20 by vol.) *p*H 6·4. Another volatile buffer, pyridine–glacial acetic acid–water (1 : 10 : 20 by vol.) *p*H 3·6, is preferred when only one-dimensional electrophoretic separation is desired. Ascending chromatography (stage 2) using the solvent *n*-butanol–glacial acetic acid–water (30 : 10 : 10 by vol.) was used by Ingram in his first investigation. Baglioni[3] has obtained improved separations with a solvent made up of pyridine–isoamylalcohol–water (35 : 35 : 30 by vol.).

The solvent butanol–acetic acid–pyridine–water (50 : 72 : 15 : 60 by vol.), as proposed by Hill *et al.*[4], has also been used with success. The disadvantage of a system with the strongly smelling pyridine is that it requires special conditions.

In our work on animal haemoglobins we have found that buffer made from ammonia–formic acid, and a solvent made from 2,6-lutidine–water, are very satisfactory for fingerprinting of haemoglobins. Equal amounts of 1 M ammonia and 0·5 N formic acid are mixed (final *p*H 6·5). If one-dimensional electrophoretic separation is desired, ammonia (0·5 M)–formic acid (1 N) buffer of *p*H 3·6 is preferred. The buffers can be used several times, but the *p*H should be checked between each running. Whatman paper 3 *MM*, dimension 48 cm × 38 cm, corresponding to the size of the apparatus used in our work (AB Analystoknik, Vallentuna, Sweden), is dipped into the buffer and excess liquid removed by blotting firmly between sheets of dry filter paper. The paper is then placed on a horizontal siliconized glass plate, which is cooled by water. After application of the sample the moist paper is covered with a sheet of polyethylene, and a weighted glass plate. Since the apparatus consists of two electrocells two papers could be run at the same time. A voltage of 30 V/cm is applied for 2·5 h. The papers were dried at 60° in a current of air for not less than 1 h.

Descending chromatography in 2,6-lutidine–water (122 : 60 by vol.) was carried out for about 20 h. Since the solvent is running rather fast (3·5 cm/h) the bottom edge of the paper is serrated with scissors as this assists even flow of the solvent off the end and helps to straighten any irregular solvent front. The paper is again dried in a current of air at 60°. The peptide spots were revealed by dipping the paper into 0·2 per cent ninhydrin in acetone, and allowing development to take place at room temperature or in a warm place not more than 40°. When a specifically staining test was needed, the ninhydrin colour was blanched by dipping the paper in a solution of 1 N hydrochloric acid–acetone (1 : 4)[3] followed by development with the reagents for sulphur[5], tryptophan[6] and histidine[7]. Alternatively, the sequence ninhydrin, sulphur and tyrosine reagent[8] was used.

In Fig. 1 are shown the fingerprints of tryptic digests of cattle haemoglobin *F* and *B* obtained with this system. More than 20 peptides can be seen. The details of the result will be given elsewhere[9].

Fig. 1. Photograph of fingerprinting of cattle haemoglobin *B* and *F* in ammonia–formate buffer, *p*H 6·5, and 2,6-lutidine–water solvent

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PATHOLOGY

Blood Group Secretion Factors in Bronchogenic Carcinoma

As a continuing study of our original report which dealt with investigations of salivary blood group secretion in metastatic cancer patients[1], we undertook an analysis of the frequencies of secretion (*Se*) and non-secretion (*sese*) related to particular cancer sites. This approach has been used by Desai and Creger[2] in gastric cancer and by several investigators in benign disease conditions[3–5]. The original premise, as extensively used in blood types and disease analyses[6], is that these genetic factors will remain stable and not fluctuate during a disease process.

As the numbers of cancer cases increased in the various categories of organ sites, our attention was drawn to the high secretion (*Se*) rate among the bronchogenic cancer patients. This selection of secretors did not appear in the other high-incidence tumours such as colon and breast cancers. We thoroughly reviewed, therefore, all the bronchogenic tumours in this series to eliminate any possible metastatic lesions and found 88 primary bronchogenic carcinomata falling into the three main histological divisions of squamous, undifferentiated and adenocarcinoma. Whether metastases had occurred or not did not concern us in this investigation since our observation was designed only to record ABO blood groups and the saliva secretor or non-secretor factors (*Se* or *sese* respectively).

Table 1 shows the secretor status of patients with the three histological types of bronchogenic tumours. According to the usual distribution of secretion of ABH in the saliva, 20·2 of these 88 patients should have been non-secretors, whereas only five appeared. One of the five non-secretors had a bronchogenic adenoma of questionable malignancy, but was included in order not to show any bias. The scarcity of non-secretion in each histological group shows that there is no selection for one type. A χ^2 test of significance reveals a high value when compared with our control series and indicates a probability inconsistent with a chance occurrence. The number of tumours in each histological group is comparable with a large national series[7], and, therefore, supports the assumption that our case distribution is within reasonable bounds of experience gained from other studies.

Table 2 gives the ABO blood groups of lung cancer and control cases. Although an excess of group A individuals and fewer group O individuals were afflicted with bronchogenic carcinoma compared with controls, the χ^2 test does not reveal a significant deviation from the controls. Similar results have already been recorded by McConnell *et al.*[6], but their excess of A was only noted in cases of undifferentiated bronchogenic carcinoma.

Table 1. Secretion Factors in Histologically Proved Primary Bronchogenic Carcinoma

Source	Histological type: Squamous *Se*	Squamous *sese*	Undifferentiated *Se*	Undifferentiated *sese*	Adenocarcinoma *Se*	Adenocarcinoma *sese*	Total
Holy Cross	10	1	13	0	9	1	34
St. Mark's	23	2	23	1	5	0	54
Total	33	3	36	1	14	1	88

Se, secretor, *sese*, non-secretor.

	Bronchogenic cancer	Control*
Secretors	83	721
Non-secretors	5	223

$\chi^2 = 13{\cdot}10$ or $P = 0{\cdot}001$.
* 944 normal controls as published (ref. 1).

Table 2. Comparison of Blood Groups of Cancer of Lung and Controls

	Cancer No.	Cancer Percentage	Control No.	Control Percentage
O	34	39	265	46
A	42	48	222	38
B	7	8	67	12
AB	4	5	24	4
Total	87	100	578	100

$\chi^2 = 1{\cdot}68$, $P = 0{\cdot}1$.

This finding of excess secretion of blood group substances among patients with bronchogenic carcinoma seems to parallel to some extent the findings of Desai[2] in patients with stomach cancer. Both mucosal surfaces are potent secretors of blood group substances whereas, in contrast, we do not obtain a significant deviation in colon cancer where the mucosa is low in blood group substance. Two possible explanations immediately occur: (1) that water-soluble blood group substances attract or alter carcinogens, favouring neoplasia or, perhaps, (2) that Lewis substance, the material produced in greater quantity by non-secretors, might reject, because of its chemical configuration, a carcinogen making it less effective.

Another interesting possibility lies in the known content of sialic acids in blood group substances. This component of mucopolysaccharides adheres closely to cell surfaces and is known to affect the 'stickiness' of cells[8]. A combination of sialic-acid-containing mucoids, which are known to be elevated in lung cancer[9], and a carcinogen could conceivably alter the mucosal cell surface by prolonged stimulation bringing about dysplastic and neoplastic changes. Whatever the actual mechanism involved, this finding offers a valuable lead in the future investigation of bronchogenic carcinoma.

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Induction of Intestinal Tumours by *N*-Ethyl-*N*-nitrosourethane

Intestinal tumours, though very rare in laboratory rodents, have been induced by several types of carcinogenic agents, including polycyclic aromatic hydrocarbons, 4-dimethylaminostilbene, 2-acetyl-aminofluorene and radiation[1,2]. However, so far, no such tumours have been induced by alkylnitroso-compounds, which are able to induce tumours in many organs[3]. Attempts to induce tumours of the colon by repeated rectal application of diethylnitrosamine led to liver, but not to local, tumours[4].

It is of interest, therefore, that when *N*-ethyl-*N*-nitrosourethane (ENU) in 50 per cent aqueous ethanol was given to rats, males and females, by intraperitoneal injections, 3 out of 6 rats which survived more than a year developed adenoma and adenocarcinoma of the mucosa of the ileum. The tumours were multiple and were found in two male and one female rat, killed 16·5, 20 and 18 months after the first and 6, 9·5 and 7·5 months, respectively, after the last of four doses of ENU, about 20 mg per rat, *in toto*. The tumours formed large cauliflower-type nodules, up to 1·5 cm diameter, obstructing the lumen, and caused enormous distension of this part of the intestine. Some of the nodules penetrated into the muscle layer of the intestinal wall. *N*-Methyl-*N*-nitrosourethane, the methyl-homologue, is only now being tested by the intraperitoneal route in rodents.

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BIOLOGY

Influence of Hexadecane on Absorption of Carbon Dioxide by Plants

Results of recent investigations concerning the influence of hydrocarbons and a petroleum oil on apparent photosynthesis in *Citrus sinensis*[1] and *Bauhinia blakeana*[2] have focused my attention on the significance of stomatal distribution in a plant's response to the application of hydrocarbons. For example, using infra-red carbon dioxide analysis with sequential multi-point sampling[3], it has been shown that an application of a paraffinic hydrocarbon to either the upper or lower surface of the leaves of *Bauhinia blakeana* results in a decrease of absorption of carbon dioxide while a similar application to the leaves of *Citrus sinensis* results in a decrease of absorption of carbon dioxide only when applied to the lower surface of the leaves. Since these results are corollary to the distribution of stomata in these species they suggest penetration only through stomata with consequential interference of absorption. The present investigation was initiated in order to clarify further the role of stomatal distribution.

With the following exceptions, infra-red carbon dioxide analysis as previously described[3] was used. Leaves of *Heliconia humilis* were enclosed in water-cooled plastic chambers which permitted sequential sampling for 5-min periods from either the lower or upper surfaces. The leaves were sufficiently illuminated from above to obtain maximum apparent photosynthesis (carbon dioxide absorption) from both surfaces. After a pattern of carbon dioxide absorption was established for both surfaces, applications of hexadecane were made using a settling tower loaded from an aerosol container. The amounts of hexadecane were determined gravimetrically.

The result of an initial application of 79 μg/cm² on the upper surface is shown in Fig. 1*a*, while the result of an initial application of 126 μg/cm² on the lower surface is shown in Fig. 1*b*. For clarification the dark periods (respiration or carbon dioxide evolution) have been omitted. Corrections have been made for minor fluctuations of carbon dioxide in air.

Initially, and as anticipated from the distribution of stomata in this species, approximately 20 times as many on the lower surface as on the upper, a greater amount of carbon dioxide is removed from the air stream passing over the lower surface[4]. With the application of hexadecane to either surface a depression of carbon dioxide absorption is obtained. It should be noted that the application to the upper surface did not interfere with absorption from the lower surface; and, conversely, the application to the lower surface did not interfere with absorption from the upper surface. These results suggest the lack of complete hydrocarbon penetration at the levels used.

Recovery from the application appears to correlate with the dissipation of the hydrocarbon. Complete recovery from the application applied to the lower surface occurred in about 6 h, while complete recovery from the application to the upper surface was not apparent until after stomatal opening the following morning. The variance between the time of complete evaporation of the hydrocarbon from the aluminium paper strips and complete recovery of carbon dioxide absorption can undoubtedly be attributed to the 'threshold levels' that result from sorption.

The results unequivocally demonstrate the significance of stomatal distribution in a plant's response to the

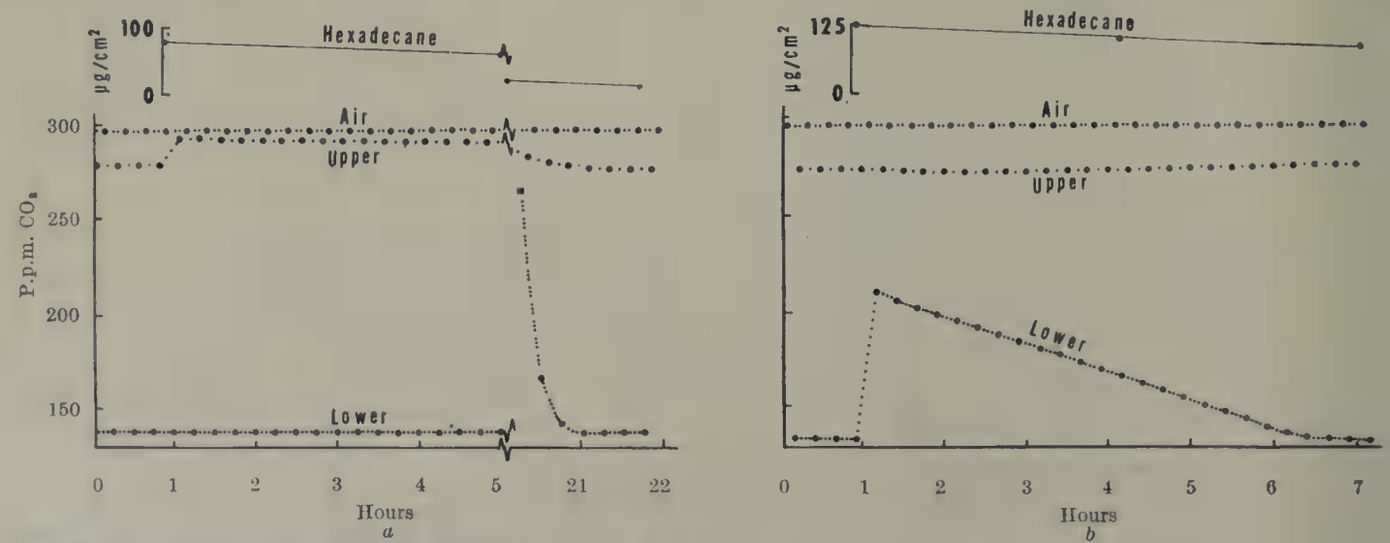

Fig. 1. Concentration, p.p.m. of carbon dioxide in air stream from 3 channels sampled sequentially. Each large dot represents one 5-min sampling period. Solid line represents evaporation of hexadecane from aluminium paper strips, μg/cm². *a*, Application to upper surface; *b*, application to lower surface. Dark periods not shown

application of hydrocarbons along with the nature and the amount of hydrocarbon applied. They also suggest, since in these experiments no visual injury was apparent, a more critical definition of 'phytotoxicity'.

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Social Companions and the Mother–Infant Relationship in Rhesus Monkeys

The development of behaviour in a young primate is influenced by both the physical and social characteristics of its environment. Paramount among the latter is the mother[1], while later other infants are important as play companions[2]. Previous investigations[3,4] of group-living rhesus monkeys suggested that adult females, other than the infant's own mother, might also play an important part, both by themselves showing maternal, social, play, aggressive and other types of behaviour towards the infant and by influencing the nature of the mother–infant relationship. For example, one adolescent female which, with the male's support, frequently interacted with the two infants in the same group, made their mothers extremely restrictive. In consequence, the infants lived confined lives: while seven other group-living infants were recorded more than 2 ft. from their mothers in a mean of 37 per cent of our observation periods when 17–18 weeks old, these two practically never went this far from their mothers up to this age.

In the experiment recorded here we attempted to assess the extent to which such a factor operates in other group-living infants where the effect is not so obvious. Nine mother–infant pairs each living in groups of a male, three or four females, and their young, were compared with four mother–infant pairs living alone. In both cases the cages were 18 ft. × 10 ft. × 8 ft., communicating with an indoor room 6 ft. × 6 ft. × 8 ft. (ref. 5). Routine watches were made between 0900 h and 1300 h G.M.T. or B.S.T. for at least 6 h a fortnight until the infants were a year old. Data were recorded on check sheets by 0·5 min periods. The levels of significance are based on the Wilcoxon matched-pairs signed-ranks test, 1-tailed, unless otherwise stated.

Differences between the group-living and the isolated mother–infant pairs were of two main types. First, the isolate infants ranged more freely. They spent less time on the nipple, less time on their mothers but off the nipple, more time off their mothers (Fig. 1), went to a distance of 2 ft. from their mothers more often, and spent longer bouts off their mothers (all $P < 0{\cdot}005$). An increase in distance between mother and infant from less than 2 ft. to more than 2 ft. was more often due to the mother moving away in the isolates than in the group-living pairs, and a decrease was less often due to the mother (both $P < 0{\cdot}005$).

A second group of differences between isolate and group-living mother–infant pairs we ascribe to the absence of other social companions for the former. Thus, although the isolate infants went to a distance from their mothers more frequently than the group-living ones, the number of 0·5 min periods spent wholly more than 2 ft. from their mothers during the second half year was less. Similarly, the frequency with which the 2-ft. radius around the mother was crossed was greater for the isolates (both $P < 0{\cdot}005$). The isolate infants thus returned to the proximity of their mothers more often. The proportion

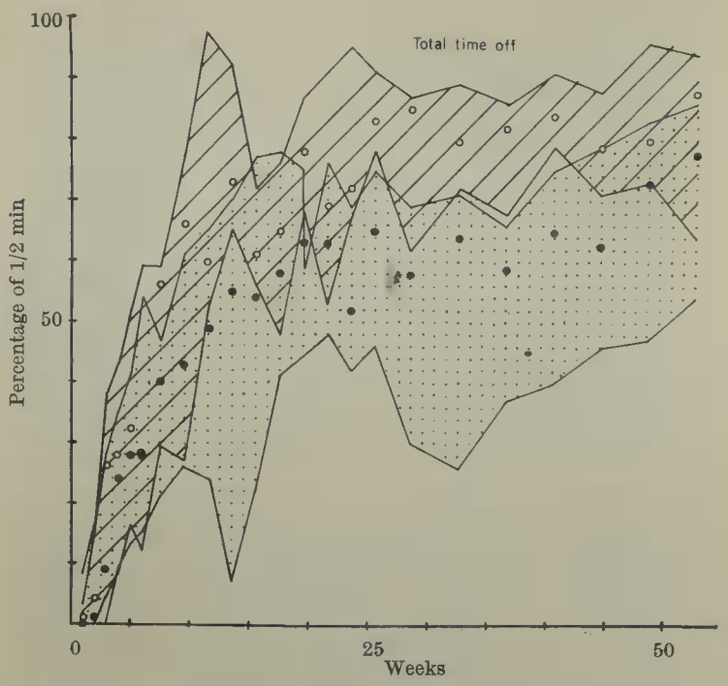

Fig. 1

of attempts made by the infants to attach to the nipple which led to acceptance by the mother was lower for the isolates ($P < 0{\cdot}04$, 2-tailed sign test). Furthermore, grooming of both infant by mother ($P < 0{\cdot}005$) and mother by infant (not significant) was higher for the isolates.

The results thus indicate that the relationship between rhesus mother–infant pairs is influenced in at least two ways by other members of the group. The presence of other adult or adolescent females results in the young infant ranging less freely from its mother, the effect being mediated largely through the mother's behaviour. The presence of social companions results in the infant returning to its mother less often than it would in their absence, and in consequence being rejected by her less often.

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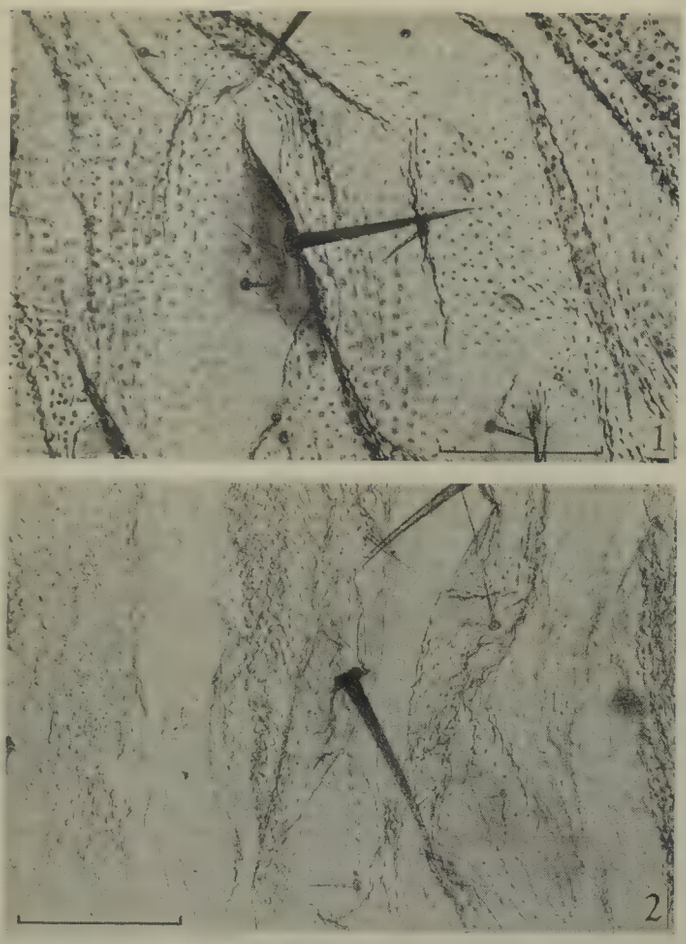
ENTOMOLOGY

Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta)

Bilateral asymmetry is virtually unknown among insects, other than as a rare aberration. Pasteels[1] has briefly noted the occurrence of colour asymmetry in larvae of *Arge ustulata* (L.) in Belgium and the Tyrol. Intensive collecting in south-eastern England and north-western Scotland has shown British populations of *A. fuscipes* (Fallén) and *A. ustulata* feeding on birches (*Betula pendula* Roth. and *B. pubescens* Ehrh.) to consist of symmetrical and asymmetrical individuals. Both forms appear to be present in proportions too large to be attributable to recurrent mutation alone. Unfortunately, however, samples from particular localities have so far been too small to allow accurate frequency determinations. Nevertheless, assuming genetic control,



Figs. 1 and 2. Flat mount in 'Euparol' of cast cuticle of asymmetrical last instar larva of *A. fuscipes* showing detail of cuticle of first abdominal segment. Fig. 1, part of darker left side. Fig. 2, corresponding part of right side. Scale, 0·2 mm

this phenomenon would appear to constitute a true polymorphism (Ford[2]).

The symmetrical larvae of both *A. fuscipes* and *A. ustulata* are pale green on both sides, while the asymmetrical forms have one side distinctly darker green than the other. In all British examples so far examined the left has been the darker side, contrasting with the condition described by Pasteels[1] from continental examples in which the right was always the darker. The asymmetry seems to be a property of the cuticle alone. In our material the deposition of melanic pigment was heavy in the cuticle of the left side but negligible on the right side (Figs. 1 and 2). On the dark side the pigment is concentrated into small, closely scattered, spots which appear to have coalesced to form larger areas at the bases of the major bristles (Fig. 1). This distribution of melanin has the effect of darkening the outward appearance of the underlying green pigment.

The larvae of *A. fuscipes* and *A. ustulata* are leaf edge-feeders, particularly on birches in Britain. Asymmetrical larvae have always been found in the field feeding with their darker left sides uppermost, and they will readopt this attitude after being disturbed. The food-plant leaves are considerably darker green above than below so that an asymmetrical larva correctly oriented is remarkably cryptic to the human eye when viewed from either side. In view of the selective advantage this type of asymmetry would appear to confer on edge-feeding larvae, it is remarkable that it is such a rare phenomenon. Even in *Arge* larvae presumably there must be some associated disadvantages which serve to maintain the polymorphism (Ford[3]).

The situation is further complicated by taxonomic difficulties. *A. fuscipes* and *A. ustulata* are very closely allied forms and their larvae cannot at present be separated with certainty. Preliminary breeding experiments suggest that the two 'species' may be only seasonal forms of a single species. It is hoped to carry out larger-scale breeding work with material from different areas in order to solve the taxonomic problem. Genetic analysis by means of large-scale rearings is also urgently needed. It should be noticed that the adult sawflies show no obvious asymmetry whatever their larval type.

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Feeding Mechanism of Blood-sucking Arthropods

It now appears to be established[1–6] that blood-sucking arthropods feed, in the main, either from the lumen of a blood vessel or from a blood pool resulting from the laceration of blood vessels. Occasionally an insect which is a vessel feeder will take part of its nourishment from a blood pool; but, except for the mosquito, this appears to be of unusual occurrence. *In vivo* observations on non-sensitized hosts have shown that a considerable number of argasid and ixodid ticks, at least one horsefly and the tsetse fly are pool feeders, while several fleas, three species of triatomine bugs and the bed-bug are vessel feeders. The present consensus of opinion is that the mosquito is largely a vessel feeder, although Gordon *et al.*[1] give reasons for believing that a proportion, at least, of the insect's blood meal is derived from haemorrhages formed in the tissues.

While the list of arthropods on which investigations of this nature have been carried out may look imposing, there are, nevertheless, some significant gaps in our knowledge. Hitherto, for example, no information has been available for such medically important insects as the stable-fly, biting midges, simuliid flies and lice. It is in order to provide some of the answers that the investigations on the stable-fly (*Stomoxys calcitrans*), the valley black-gnat (*Leptoconops torrens*) and the hog-louse (*Haematopinus suis*), summarized in this communication, have been undertaken.

These investigations have shown that both the stable-fly and the valley black-gnat, on laboratory white mice, are true pool feeders. The stable-fly possesses a proboscis which is not unlike that of the tsetse-fly but the labellar teeth are much coarser. The proboscis is introduced into the tissues somewhat like a cylindrical drill, the stout labellar teeth producing a haematoma from which the fly feeds. On the other hand, the feeding mechanism of the valley black-gnat generally resembles that of the horse-fly, *Haematopta pluvialis*. The mandibles cut the tissues with a scissor-like movement while the maxillae move rapidly to and fro.

Observations on *Haematopinus suis* were carried out on the ear of a laboratory white mouse. Following the introduction of the proboscis into the skin, the haustellum is everted and the strikingly flexible stylet bundle probes the tissues until a suitable blood vessel is located. This work has shown unequivocally that the hog-louse is a true vessel feeder.

Arthropods which feed from small vessels have hitherto been known as 'capillary feeders'[1]. The term 'capillary feeder' cannot, however, be regarded as strictly correct if the capillary is defined as a small vessel of approximately 7μ in diameter[7], since observations on feeding carried out over the past few years have shown that feeding is not generally from capillaries but usually from venules or small veins. The term 'vessel feeding' should replace 'capillary feeding' before usage fossilizes the expression.

Finally, attention needs to be directed to the difficulty experienced in translating succinctly into certain foreign languages the expressions 'pool feeder' and 'vessel feeder'; either the term must be directly borrowed from English, which is not always satisfactory, or else requires cumbersome translating. The introduction of two terms derived from Greek roots is therefore proposed for more universal application, 'solenophage' (from the Greek σωλήν = pipe and φαγο = eating) for vessel feeder, and 'telmophage' (from the Greek Τέλμα = pool and φαγο = eating) for pool feeder.

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MICROBIOLOGY

Metabolism of Nucleic Acids and Protein in Starving Bacteria

During an investigation of metabolic processes in starving bacteria we observed that a significant net synthesis of deoxyribonucleic acid (DNA) took place in them[1]; no increase in the amount of ribonucleic acid (RNA) and protein could be detected (unpublished results).

The procedure in the foregoing experiments was as follows. Log-phase cultures of *Escherichia coli B* grown in a minimal medium were collected by centrifugation at an optical density of about 0·200, washed and re-incubated in acetate buffer (*p*H 7) at 37° C with aeration. Samples for biochemical analysis were taken immediately after resuspension and at the time intervals indicated in Fig. 1.

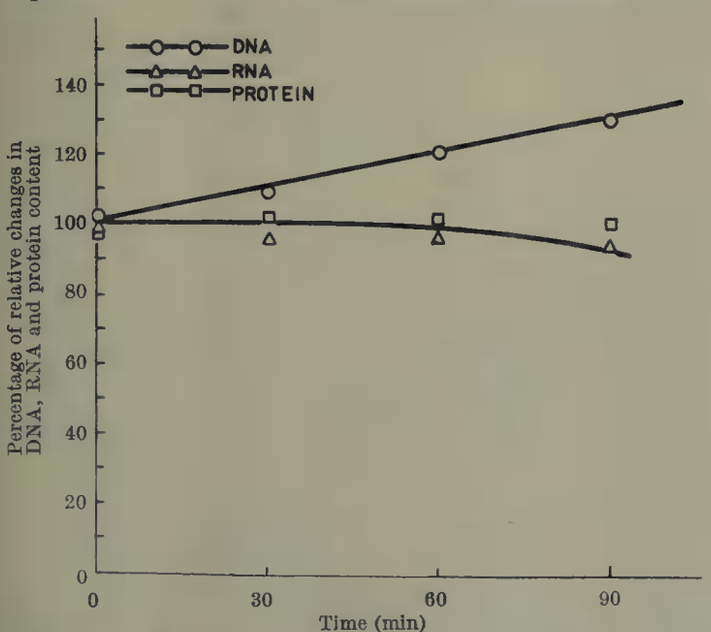

Fig. 1. DNA, RNA and protein content in *Escherichia coli B* incubated in acetate buffer. The content of these macromolecules in the bacteria at 0 min is taken as 100 per cent

Chemical fractionation of the bacteria, as well as the determination of DNA, was carried out by Burton's method[2], RNA was determined according to Mejbaum[3], and protein as described by Lowry *et al.*[4].

As seen in Fig. 1, the amount of DNA increased during 90 min incubation by about 30 per cent, while no biosynthesis of RNA or protein could be observed. Thus we postulated that the nucleic acid's precursor pool must be in this case preferentially utilized for DNA synthesis which is, consequently, favoured in some way in the starving bacteria.

To verify this hypothesis on a similar but more specific system, we used a 'uracil-less' mutant of *E. coli B*, which is capable of synthesizing only about 5 per cent of RNA and protein if no uracil is added to the growth medium.

E. coli B U⁻ was incubated in '3 × *D*'-medium[5], to which 30 μg/ml. uracil was added. At an optical density of about 0·150, 0·02 μc./ml. of ^{14}C-uracil (specific activity 6·5 μc./μmole) was added to the suspension, and the incubation was continued for a further 2 min. The suspension was then quickly chilled, centrifuged in the cold, and washed twice by cold physiological saline. The bacteria were resuspended at the same optical density in cold '3 × *D*'-medium containing no added uracil, and re-incubated at 37° C with aeration. Samples for analysis were taken immediately after resuspension and after 10, 20 and 30 min incubation.

Chemical fractionation was carried out by a somewhat modified form of the Schmidt–Tannhauser method[6]. The radioactivity of the acid-soluble, as well as of the DNA- and of the RNA-containing fractions, was counted in a gas flow counter on planchets, after having dried on them 200 μl. of the corresponding extracts.

The results are summarized in Table 1. It is evident that there is a steady increase in the radioactivity of the DNA-containing fraction, while the activity of the acid-soluble fraction decreases rapidly. There is also a temporary increase of the radioactivity of the RNA-containing fraction, which, however, is not very significant if the initial high activity of RNA is taken into account (see data under *B* in Table 1).

Table 1. Percentage of Radioactivity from ^{14}C-Uracil in the DNA, RNA and Acid-soluble Fractions

Fraction	0 min		10 min		20 min		30 min	
	A (%)	*B*	*A* (%)	*B*	*A* (%)	*B*	*A* (%)	*B*
DNA	5·1	100	9·5	186	12·4	243	13·9	272
RNA	80·7	100	88·0	109	84·3	104·5	84·0	104
Acid-soluble	14·2	100	2·5	17·6	3·3	23·2	2·1	14·8

A, Percentage of total radioactivity from all three fractions.
B, Relative change in radioactivity, compared with counts at 0 min

Table 2

Fraction	0 min		20 min	
	*A**	*B*	*A*	*B*
DNA	8·4	100	16·8	200
RNA	72·0	100	80·7	113
Acid-soluble	19·6	100	2·5	13

**A* and *B* as in Table 1.

Data comparable with those in Table 1, but obtained in another experiment of the same type, are shown in Table 2. In this experiment DNA was isolated after 20 min incubation of the bacterial culture, by Spirin's method[7], and hydrolysed by 72 per cent perchloric acid, the bases being isolated and separated by paper chromatography[8].

After elution of the ultra-violet-absorbing spots, measurable radioactivity was found only in the spots with the R_F corresponding to those of cytosine and thymine, the specific activities (c.p.m./μmole) being: for cytosine, 0·929; for thymine, 0·790.

The presence in thymine of radioactivity comparable with that of cytosine indicates that the radioactivity from ^{14}C-uracil was indeed incorporated into DNA molecules. This incorporation proceeds in spite of the very limited endogenous pool of uracil and goes on even when incorporation into RNA stops altogether.

These data seem to support our assumption as to the preferential utilization of nucleic acid precursors for DNA

synthesis in bacteria, incubated under starvation conditions or in a deficient medium.

The starving bacteria, incubated in acetate buffer, contain also an amino-acid pool which is, however, not utilized for protein synthesis, as can be seen in Fig. 1. This observation may point to the possibility that the limited potentiality of utilization of energy for anabolic processes in the starving cells is also oriented in the first place toward DNA synthesis. If this assumption was also shown to be correct, it would indicate that in our systems the kinetics of biochemical reactions on the precursor's level are such that processes leading to DNA synthesis are favoured over those leading to the synthesis of other macromolecules.

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VIROLOGY

Transmission of Guamá and Oriboca Viruses by Naturally Infected Mosquitoes

Most of the present knowledge about the ability of mosquitoes to serve as natural hosts for arthropod-borne viruses is based on two methods. The first consists of triturating the wild-caught mosquito and inoculating it into a laboratory animal; subsequent virus isolation proves natural infection of the mosquito but not ability to transmit. The second consists of infecting the mosquito on a viraemic laboratory host and demonstrating transmission by bite to another laboratory host. This method demonstrated the ability to transmit, but may not reflect what happens under natural conditions. The technique described here makes possible a demonstration of transmission by naturally infected arthropods.

Mosquitoes captured during the first half of 1964 in mouse- and chicken-baited traps in the Instituto Agronomico do Norte forest near Belém, Brazil, were identified while alive and liberated in screened holding cages (100 cm × 100 cm × 115 cm) in the forest. A separate cage was set up for each mosquito species examined. Families of 3-day-old Swiss mice with the mother mouse were placed in the cages to provide a blood meal. These mice were observed for subsequent illness on the chance that the wild-caught mosquitoes might have been naturally infected with arthropod-borne viruses and have transmitted them to the mice.

Between February 24 and June 30, 2,846 *Culex* (*Melanoconion*) *taeniopus* females were released in their cage and 68 families of mice exposed, each for a 24-h period. Guamá virus was isolated from the blood of a mother mouse exposed on April 14.

Between January 30 and June 30, 2,860 *Culex* (*Melanoconion*) females, of a species morphologically similar to '*Culex* No. 9'[1] of Trinidad, were released in another cage and 109 families of mice exposed. Oriboca virus was isolated from a baby mouse exposed on May 9.

The transmission of Guamá and Oriboca viruses to Swiss mice by naturally infected *Culex* (*Melanoconion*) has thus been demonstrated. This same technique was used by Sérié *et al.*[2] to transmit yellow fever to laboratory mice by the bite of naturally infected *Aedes simpsoni* in Ethiopia.

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CYTOLOGY

Circulation in the Cell

The rapidity with which cells respond to the presence of solutes or suspensoids in their immediate vicinity has made the problem of the permeation of the cell a matter of major interest, but the question of how substances are distributed once they have entered the cell has not commanded nearly so much attention. I shall propose that the rapid distribution of nutrilites and reactants within the microcosm of the protoplast (that is, inside the plasma membrane) is effected by an active circulatory apparatus, by which fluid is forced through pores by hydrostatic pressure, somewhat analogous to the apparatus by which a corresponding circulatory distribution is effected in the macrocosm of the animal body. The concept of the cell-circulation is based almost exclusively on work with the yeast cell; but there are many indications which suggest that the conclusions are generally applicable. Many cellular membranes, which were previously thought to be intact, have been shown by electron microscopy to possess systems of pores. Pores which have been first observed by electron microscopy have been afterwards observed by direct light microscopy.

The circulation-hypothesis explains the rapid distribution of cell-substances which is inconsistent with a diffusion hypothesis. The fact that cells continuously change in their ability to take up substances from the external *milieu* is indisputable. It seems unrealistic, however, to try to account for such changes by changes in the composition of the membrane because a minor change, which might accommodate one substance, would certainly alter the behaviour of the membrane to a variety of other substances. It seems essential that any hypothesis should require that the structural integrity of the membrane-systems should remain unaltered throughout the life of the cell. It is interesting in this connexion that Lehninger[1] has suggested: (*a*) that membranes are usually assembled *in situ*; (*b*) that protein synthesis is concerted with phospholipid synthesis in such a manner that the two syntheses are mutually dependent; (*c*) that membrane synthesis occurs by a process in which the lipid and protein components of the membrane serve as 'structural templates' for each other, to form a thermodynamically stabilized end-product; (*d*) that each membranous structure may produce new membrane from proteins and lipids present in the protoplasm by using the pre-existing membrane as a template.

Although genes might change by mutation to produce enzymes which might be able to synthesize new membrane-components which might be able to alter the selective permeability of the membrane, it seems unlikely that changes in permeability are primarily due to such a mechanism. It seems much more likely that genes controlling the entry of a substance into the cell do so by eliciting the production of an enzyme which converts the substance into a form which can pass through the cellular membrane. According to the receptor hypothesis[2], genes act only in response to the contact stimulus of an inducer. As a general corollary to this hypothesis, a large molecule which might not be able to pass through the intact barrier of the plasma membrane and which could not enter any 'normal' membrane in metabolically

significant amounts (that is, in amounts detectable by changes in oxygen consumption and carbon dioxide production) might, nevertheless, be assumed to act as an inductor of enzyme at the molecular level. Minute amounts might be able to enter the cell-circulation by imperfections (due to pinocytotic pores or fissures) which could act as inductors of enzymes by making contact with the gene-receptor in amounts which might not be detectable by metabolic measurements.

On the circulation theory the permeability of each of the cell membranes is assumed to remain virtually unchanged throughout the metabolic processes, and no enzymes are assumed to be elicited which change the permeability of the membrane *per se*. This theory does not require that permeation be purely by diffusion nor exclude the possibility that permeation may be facilitated by specific energetic mechanisms. It is assumed that adaptive permeation in the yeast cell is usually effected by contact of large molecules with hydrolytic enzymes which have been induced by a few molecules of the inductor and that the enzymes are afterwards expelled into the cell wall, or the nucleoprotein layer. In the nucleoprotein layer[3] the extra-protoplastic enzymes, which were induced in the cell by adaptive processes, hydrolyse large molecules into smaller ones which move much more rapidly through the plasma membrane, possibly by facilitated transport. The nucleoprotein layer performs the same function for the yeast cell that the small intestine and the lymphatic system perform for the vertebrate organism.

After the plasma membrane has been passed and the substance has entered the cytoplasm, the cell circulation rapidly distributes the materials through the cytoplasm and inside the nuclear apparatus. The 'pump' controlling the cell circulation is located in the nuclear apparatus and establishes a flow from the cytoplasm into the interior of the nucleus that ensures a relatively rapid movement of materials from the external *milieu* to the surface of the chromosomes. The motive power for the pump is furnished by the 'pumping units' of Oestergren[4], who proposed that the spindle is coated with units which carry on a 'pumping activity' by which the chromosomes are moved to the poles. Oestergren *et al.*[4] have suggested that substances are transported on and within the spindle by a pumping activity which drives materials from the equator in the two opposite directions while the spindle substance remains stationary. The pumping activity carries both chromosomes and acentric bodies to opposite poles of the cell much as sailboats are driven by the wind. I have assumed[5] that the pumping units ('spindle-micellae') are stored in the spindle reservoir of the yeast cell (which corresponds to the resting telophase nucleus) enclosed within the nuclear membrane and oriented to pump cytoplasm through the nucleus over the surface of the diffuse chromosomes. In the yeast cell an exit port leads from the spindle reservoir into the nuclear vacuole and hydrostatic pressure inflates the vacuole to an almost perfect sphere. Two exit (extruder) ports in the vacuolar membrane permit the slow discharge of a ribbon of endoplasmic reticulum, the attached ribosomes (carrying messenger RNA) and other solid materials from the nuclear vacuole into the cytoplasm[6]. But the solutes, especially the methylated nucleotides of the nucleolus, from which the transfer RNA is synthesized in the cytoplasm (on the surface of the mitochondria) pass rapidly through the membrane of the nuclear vacuole into the cytoplasm on the stream which enters the cytoplasm through this thin, readily permeable barrier. The membrane of the spindle reservoir is impermeable to nucleotides.

Fig. 1 shows the 'flow sheet' of the cell. (1) The cell wall is composed largely of polymers of hexoses or hexose derivatives. (2) The nucleoprotein layer is confined between the coarse network of the cell wall and the much less permeable plasma membrane and contains an abundance of a variety of enzymes which were produced inside

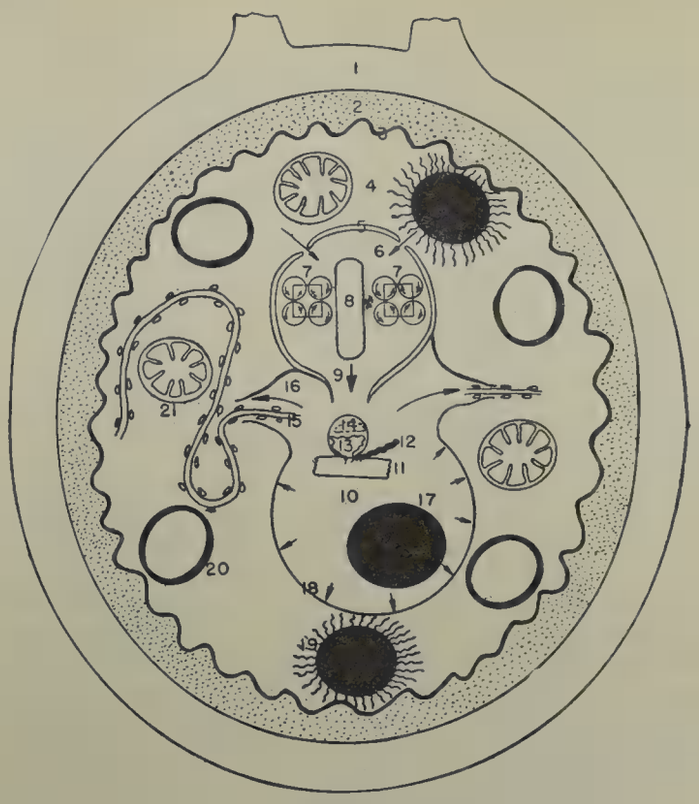

Fig. 1

the cell. (3) The plasma membrane is a typical, protein-phospholipid, biological membrane, impermeable to most sugars larger than hexoses. (4) The cytoplasm is a limpid fluid in which the cellular organelles are suspended. (5) A thick, relatively impermeable, protein-phospholipid membrane containing both entry (6) and exit (9) ports surrounds the spindle reservoir. The spindle reservoir contains the diffuse chromosomes (8) and the pumping units (7). The diffuse chromosomes are expelled into the nuclear vacuole (10) from the spindle reservoir where they become condensed chromosomes. Each particulate gene carried on the histone backbone (11) of the chromosome contains a proteinaceous receptor (12) and a double loop of structural DNA (13) on which messenger RNA (14) is synthesized. (18) The membrane surrounding the nuclear vacuole is very thin, easily permeable and quickly destroyed by most cytological fixatives. In life, it is maintained in a condition of turgor by the hydrostatic pressure of the nuclear pump. The endoplasmic reticulum and its attached ribosomes (15) are extruded into the cytoplasm through a port (16) in the vacuolar membrane. The nuclear vacuole contains the nucleolus (17) in which the nucleotides from which transfer RNA is later synthesized are collected and methylated. The cytoplasm contains mitochondria in three different states: transfer RNA is synthesized from nucleolar nucleotides on the surface of the mitochondrion (19). In this state, the mitochondrion absorbs ultra-violet and stains with uranyl acetate. (20) The mitochondrion is the site of fat synthesis and in this condition is highly refractile in the light microscope and electron transparent. (21) The cristate mitochondrion, closely associated with the endoplasmic reticulum, is the cytoplasmic source of ATP.

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GENETICS

Schizophrenia as a Genetic Morphism

Huxley, Mayr, Osmond and Hoffer recently proposed[1] the hypothesis that schizophrenia was a genetic morphism. This was based on three main streams of evidence: (1) genetic; (2) clinical; (3) biochemical. Baldessarini and Snyder[2] criticized this hypothesis for being premature. But every hypothesis is premature, for once the basic data are well established hypotheses are not required.

As the biochemical-clinical member of our group, I will refer to certain errors in the Baldessarini critique.

The arguments on which the adrenochrome hypothesis was based depend not only on the presence of adrenochrome in tissues, but also on a very comprehensive number of facts recently reviewed by me[3]. The evidence is both indirect and direct, that is, based on the reports of many independent scientists that adrenochrome or adrenolutin was found by them in body fluids. These works have not been referred to by the two critics. Nor have they referred to work by Axelrod[4] at the U.S. National Institute of Mental Health, who demonstrated the conversion of adrenaline into adrenochrome in salivary tissue.

They further state, "It is thus quite unlikely that adrenochrome or adrenolutin are unique biochemical factors in schizophrenia". We at no time claimed these chemicals were "unique to schizophrenia", but instead attempted to show that adrenochrome was present in normal people and was diverted in greater concentration to adrenolutin in schizophrenics.

They further suggested that the mauve factor first reported by Irvine[5] and Hoffer and Mahon[6] could be related to dietary factors. Had they read Irvine it would have been apparent this was one of the remotest possibilities, since dietary factors were investigated and excluded by Irvine. The Irvine and the Hoffer and Mahon factors are essentially alike although the Irvine factor may contain more components. Both methods were cross-validated against each other. The mauve factor test has been used routinely in three psychiatric centres in Saskatchewan for up to five years, and has consistently been related only to severity of the psychiatric condition. It will, therefore, follow from the critics' position that a main variable in production of these illnesses is dietary—a novel idea.

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Identical *S* Alleles in Different Botanical Varieties of *Brassica oleracea*

Self-compatibility is rare in marrow-stem kale (*Brassica oleracea* var. *acephala*) and usually occurs only in plants which have *S* alleles low in the dominance series. In such plants, genes, independent of the *S* alleles, determine self-compatibility[1]. In other botanical varieties of *B. oleracea* self-compatibility is common and this suggests that these varieties might have the *S* alleles of low dominance found in marrow-stem kale.

Five inbred plants of marrow-stem kale, each homozygous for a different *S* allele, four of which had been identified in self-compatible plants, were crossed as males to Brussels sprouts (*B. oleracea* var. *gemmifera*), to a few plants of dwarf curly kale (var. *acephala*), savoy cabbage (var. *sabauda*), purple-sprouting broccoli (var. *italica*) and to one wild cabbage plant. Each of the five *S* alleles was identified in at least one of the botanical varieties. The allele S_2 was found in all the botanical varieties tested and was active in 19 of the 48 plants examined (Table 1); another allele, S_{15}, occurred in three of these varieties; and the five *S* alleles accounted for more than one third of the total number of *S* alleles present in these plants (35 out of 96).

Table 1. Numbers of Plants in which the *S* Allele was Active in the Stigma

Botanical variety	No. of plants examined	*S* alleles 2	5	15	26	30
Brussels sprouts	30	10	6	2	0	3
Dwarf curled kale	9	2	0	2	2	0
Purple-sprouting broccoli	5	4	0	1	0	0
Savoy cabbage	3	2	0	0	0	0
Wild cabbage	1	1	0	0	0	0
Totals	48	19	6	5	2	3

In the stigmas of plants with unidentified *S* alleles, S_2 can always be recognized because it is active in combination with each of the fifteen different *S* alleles from marrow-stem kale, with which it has been tested. In kale, S_2 is recessive in the pollen with thirteen *S* alleles, incompletely recessive with one, and active with two others very low in the dominance series. Hence, the proportion of plants with S_2 active in the pollen to those with S_2 active in the stigma indicates the dominance of the other *S* alleles in a sample. Of the ten plants of Brussels sprouts in which S_2 was active in the stigma, S_2 was also active in the pollen in six. This confirms that there is a higher proportion of *S* alleles low in the dominance series in Brussels sprouts than in marrow-stem kale. Of the four purple-sprouting broccoli plants which had S_2 active in the stigma, only one had this allele active in the pollen.

These results suggest that horticultural varieties of *Brassica oleracea* may differ from agricultural kales in having a smaller number of *S* alleles, more of which are low in the dominance series and which therefore allow a higher proportion of self-compatible plants. Such differences could be a direct consequence of the number of plants selected to produce basic stocks. A commercial variety of marrow-stem kale may be based on several hundred mass-selected plants, whereas a variety of Brussels sprouts may be derived from a few rigorously selected plants. Selection of a few plants would reduce the number of *S* alleles, and subsequent seed multiplication would favour the retention of partially self-compatible plants with *S* alleles low in the dominance series. Thus, Sampson[2] found in Calabrese green-sprouting broccoli (var. *italica*) that four plants from a population had only four different *S* alleles, and he observed that "this represents a remarkably high concentration of *S* alleles but may be due to sampling rather than to any intrinsic feature of broccoli population structure". The present results suggest that Sampson's sample may be representative of the broccoli population.

The high proportion of plants in the different botanical varieties, which have allele S_2, is surprising. It is unlikely that it has spread during the last century by cross-pollination between breeders' distinct basic stocks of cabbage, Brussels sprouts and curly kale. *S* alleles are remarkably stable (Lewis[3] found no new alleles for self-incompatibility in 22 million gametes of *Oenothera*) so that independent mutation to allele S_2 in different botanical varieties of *B. oleracea* can be ruled out. In potatoes, also, *S* alleles appear to be very stable; Dodds[4] found the same *S* alleles in diploid cultivated species and in polyhaploids of autotetraploid 'Andigena' potatoes. It is therefore probable that allele S_2 occurred in the ancestral *B. oleracea*, from which the present botanical varieties have evolved. In addition, because relatively small numbers of plants are selected to maintain basic stocks of these

varieties, it is probable that S_2 must have some inherent selective advantage.

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VETERINARY SCIENCE

Association of Adenoviruses with Bovine Respiratory Disease

A strain (*WBR* 1) of bovine adenovirus (BA) was isolated in primary cultures of bovine kidney (BK) and found to be antigenically distinct from BA type 1 and BA type 2 (ref. 1). The *WBR* 1 strain has therefore been proposed as the prototype strain of BA type 3.

To assess its pathogenicity for cattle, 8 colostrum-deprived calves were inoculated intranasally on the day of birth with 10 ml. of BK culture fluid containing *WBR* 1 strain and with a further 10 ml. intratracheally 24 h later. The inocula had a mean titre of $10^{4.0}$ $TCID_{50}$ per ml. The calves were kept individually in isolation and then killed 4 days (3 calves), 7 days (3 calves) or 3 months (2 calves) after inoculation.

During the week following inoculation, the calves showed clinical evidence of disease manifested by pyrexia and a mucoid nasal or conjunctival discharge which lasted 2–4 days. One calf was dyspnoeic and two other calves died 3 and 6 days post-infection respectively. Five of the calves also developed a diarrhoea during the time of pyrexia. Virus was recovered either from the faeces or from nasal and conjunctival secretions for up to 11 days after inoculation; on one occasion, virus was recovered from the nose of a calf at 3 weeks. At necropsy, areas of consolidation, collapse and emphysema were evident in varying degree in the lungs of each calf. In calves killed at 4 days, the lung lesions were present mostly in the hilar region, whereas in those killed at 7 days, they extended to the periphery of most lobes. There was lymphadenitis of the lymph nodes draining the pneumonic lungs. In the calves killed 3 months after infection the gross pulmonary lesions were small and discrete.

Histological examinations indicated that the prime lesion was a proliferative bronchiolitis associated with necrosis and desquamation of epithelium. These changes resulted in occlusion of the bronchioles with consequent alveolar collapse. Typical adenovirus intranuclear inclusions, in varying stages of development, were present in the lungs, tonsils and tracheal mucosa, as well as in the retropharyngeal, mediastinal and bronchial lymph nodes. In the calves killed at 3 months both the gross and histological lesions were minimal and consisted of some degree of lymphoreticular hyperplasia accompanied by patchy areas of scar tissue.

Two control calves inoculated with uninfective BK culture fluid were killed for examination after 4 and 7 days respectively. Both calves remained normal and free of virus throughout and no macroscopic or histological evidence of infection was noted in either animal.

Two further calves were killed 7 days after inoculation with a comparable dose of *WBR* 1 virus which had been pre-treated with trypsin to remove possible 'toxic' factors of the type associated with human adenovirus type 5 (ref. 2). There was a similar clinical response but, at necropsy, the gross lung lesions were minimal and comparable to those seen in the calves killed at 3 months after infection with untreated virus. Cells with inclusions were again present in the respiratory tract but were difficult to find. This finding suggests that the trypsin-sensitive factor associated with the virus assists in the production of the pneumonic lesions observed previously.

Virus was recovered *post mortem* from various tissues from all the calves inoculated with virus, including the lungs of the calves killed at 4 and 7 days or those which died, but not from the lungs of those killed at 3 months or from those inoculated with trypsin-treated virus (Table 1). Immunodiffusion tests on the sera of all the calves showed that adenovirus group-reactive precipitating antibody developed in 3–4 weeks after infection and could persist thereafter for at least 2 months.

These experiments indicate that the *WBR* 1 strain of BA type 3 is pathogenic for calves, and the results will be reported in detail elsewhere[3]. Serological evidence has been adduced previously that adenoviruses are associated with bovine respiratory disease in the field[4]. In further investigations with paired serum samples from affected or in-contact cattle, significant increases in adenovirus group-precipitating antibody have been observed in approximately 30 per cent of 150 disease outbreaks over the past 2 years[5]. The majority of such outbreaks attributed to adenovirus infection have occurred during the winter months, with peak incidence during the earlier

Table 1. Recovery of *WBR* 1 Strain of Bovine Adenovirus Type 3 from Tissues of Calves *post mortem*

Tissues	Untreated virus inoculum										Treated virus inoculum		Controls	
	4 days			7 days			3 months		Died		7 days			
	1	2	3	1	2	3	1	2	1	2	1	2	1	2
Lung lobes:														
R. apical	+	+	−	+	+	+	−	−	−	−	−	−	−	−
R. cardiac	+	+	−	+	+	−	−	−	−	+	−	−	−	−
R. diaphragmatic	+	+	−	+	+	−	−	−	+	−	−	−	−	−
Intermediate	+	+	+	+	+	+	−	−	+	−	−	−	−	−
L. apical	+	+	−	+	+	−	−	−	+	−	−	−	−	−
L. cardiac	+	+	−	+	+	−	−	−	−	+	−	−	−	−
L. diaphragmatic	+	+	−	+	+	−	−	−	+	−	−	−	−	−
Conjunctiva	−	−	−	+	+	−	−	−	+	−	−	−	NT	NT
Nasal *m/m*	−	−	−	+	+	−	−	−	−	+	−	−	NT	NT
Retropharyngeal *LN*	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tonsil	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Larynx	−	−	NT	−	+	−	−	−	NT	−	−	−	NT	NT
Tracheal *m/m*	+	+	+	+	+	−	−	−	−	+	−	+	−	−
Bronchial *LN*	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Mediastinal *LN*	−	−	−	+	−	−	+	−	−	−	+	−	NT	NT
Cardiac muscle	NT	−	NT	−	NT	−	−	−	NT	−	−	−	NT	NT
Oesophagus	−	−	−	−	−	−	−	−	NT	−	−	−	NT	NT
Abomasum	−	−	−	−	−	−	−	−	NT	−	−	−	NT	NT
Jejunum	−	−	−	−	−	−	−	+	NT	−	−	−	NT	NT
Colon	−	−	−	−	−	−	−	−	NT	−	−	−	NT	NT

LN, lymph node. *m/m*, mucous membrane. +, virus recovered. −, virus not recovered. NT, not tested.

months of the year. Available evidence suggests that significant increases in neutralizing antibodies to BA types 1, 2 and 3 occur in the sera of cattle involved in respiratory disease outbreaks in Britain[6] and that it is also possible that further, undefined serotypes of bovine adenoviruses may be involved.

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[6] Darbyshire, J. H., and Pereira, H. G. (unpublished results).

Hepatic Tumours in Ducks fed a Low Level of Toxic Groundnut Meal

The toxicity of certain samples of groundnut meal has been shown to be due to fungal metabolites, collectively known as aflatoxin, produced by strains of *Aspergillus flavus*[1]. The carcinogenic effect of such meals for rats, a species relatively resistant to the acute effect of aflatoxin, has been demonstrated[2–4] and it has since been shown that crystalline aflatoxin is carcinogenic for these animals[5,6].

Young ducklings are the most susceptible animals to the acute effect of aflatoxin[7]. This communication describes the change found in ducks fed for a prolonged period on a ration containing a low level of toxic groundnut meal.

Thirty-seven 7-days-old Khaki Campbell ducklings (group *A*) were fed a commercial poultry ration free of aflatoxin to which was added 0·5 per cent toxic Brazilian groundnut meal which contained approximately 7 p.p.m. aflatoxin (assayed as aflatoxin B_1). Sixteen control ducklings (group *B*) of the same age and breed were fed the commercial ration without added groundnut meal. All birds received tap-water *ad lib*. They were brooded for the first three weeks in electrical tier brooders and thereafter housed in arks on pasture. Weights were recorded weekly for the first 14 weeks of the experiment. Deaths in both groups were recorded and tissues retained for histological examination. The survivors in both groups were killed fourteen months after commencement of the feeding trial.

Tissues were fixed in formal-saline, embedded in paraffin wax, sectioned at 7μ and stained with haematoxylin and eosin. Liver sections were, in addition, stained with Masson's trichrome stain and frozen sections were prepared and stained with oil red O.

Plotting the mean weight of the birds in the two groups against time showed no significant differences in the slope of the two regression lines, but the differences between means for the groups were significant at week 3 (group *A* 11·6 oz., group *B* 15·2 oz. ($P < 0.05$)), week 4 (group *A* 16·0 oz., group *B* 22·3 oz. ($P < 0.01$)) and week 5 (group *A* 23·5 oz., group *B* 27·9 oz. ($P < 0.05$)) but not at any other time to week 11. From weeks 11 to 14 there was no longer a linear weight increase in either group; the controls remained at a higher mean level than the treated birds and this difference was highly significant ($P < 0.01$) on mean values alone.

During the first four weeks of the experimental period nineteen birds in group *A* died. On histological examination lesions consistent with poisoning due to aflatoxin were present in the liver of all of them although in three birds death was ascribed to other causes. In the following 7 months a further seven birds in group *A* died, or were killed, and in each of these hepatic lesions consistent with aflatoxicosis were present.

In group *B* four ducks died due to bacterial diseases and two were killed because of severe trauma inflicted by their pen-mates. On histological examinations of the livers from these birds there was no evidence of aflatoxicosis.

Fourteen months after commencement the survivors of both groups, consisting of eleven in group *A* and ten in group *B*, were killed. Macroscopic lesions were confined to the livers; those from birds in group *A* were atrophied, putty coloured and contained numerous yellowish focal lesions 1–2 mm diameter. In four of the six drakes there were, in addition, solid yellow-coloured nodules varying in size from 1 to 3 cm diameter. In four of the five ducks, in addition to the small focal lesions, there were nodules in the livers between 0·5 cm and 2·0 cm in diameter. In group *B*, comprising six drakes and four ducks, one drake had a number of small irregularly-shaped necrotic lesions in the liver. Apart from this there were no other macroscopic lesions in the organs of the birds in this group.

The small focal lesions present in the livers of all birds in group *A* were lymphoid foci composed mainly of mature lymphocytes surrounding degenerating hyperplastic bile duct epithelium. There were no mitotic figures but, peripherally, there was infiltration, in some cases extensive, of lymphocytes between the hepatic cells. The larger nodules were liver tumours of two types: thickly encapsulated, vacuolated hepatomata composed of hypertrophic ballooned cells occasionally containing giant vesicular nuclei with very prominent nucleoli, and cholangiomata composed of dense masses of well-differentiated bile ducts (the cells of which showed very few mitotic figures) surrounded by a thin fibrous capsule. This variation in cell type is seen in hepatic tumours in rats fed aflatoxin and other carcinogens[5]. In the liver of one duck tumours of both types were present, in three ducks and two drakes there were hepatomata, and in two of the other drakes cholangiomata occurred.

After 14 months eight of eleven birds fed a ration containing 0·5 per cent toxic groundnut meal developed hepatic tumours whereas none occurred in ten birds fed the same ration without groundnut meal. The aflatoxin B_1 content of the tumour-inducing ration for ducks was approximately 0·03 p.p.m. compared with 0·4 p.p.m. required to produce hepatic tumours in rats within 13–18 months[5]. The single oral LD_{50} dose of aflatoxin for male rats is 7·2 mg/kg[8] compared with 0·4 mg/kg for male ducklings[9]. The relative susceptibility of ducks and rats to both the acute and hepato-carcinogenic effect of aflatoxin is therefore comparable.

I thank Dr. J. G. Campbell of the British Empire Cancer Campaign for Research, Edinburgh, for his advice and Miss C. N. Hebert of this Laboratory for statistical analysis.

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[8] Butler, W. H., *Brit. J. Cancer*, **18**, 756 (1964).
[9] Carnaghan, R. B. A., Hartley, R. D., and O'Kelly, J., *Nature*, **200**, 1101 (1963).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 18

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. A. Gill: "Impact Testing and Brittle Failure".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. B. A. D. Stocker: "Salmonella Antigens—Structure and Genetics" (further lecture on October 25).*

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Prof. B. D. Spalding: "Heat Transfer Aspects of Coal Utilization" (Fourteenth Coal Science Lecture).

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. H. Hamence: "The Public Analyst's Approach to Pesticide Residues".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Whither Small V-form Diesel Engines?"

ROYAL INSTITUTE OF CHEMISTRY (at Enfield Technical College, Queensway, Ponders End), at 7 p.m.—Mr. W. G. Busbridge: "Isotopes in Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Charles Swithinbank: "A Year with the Russians in Antarctica".

Tuesday, October 19

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. D. McNally: "Are Stars Possible?"*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Sources of Understanding: Is Observation Enough?"*

UNIVERSITY OF LONDON (at the Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Mr. John Maddox: "The Nuffield Foundation Science Teaching Project".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. C. D. Brown: "Design and Construction of the George Street Bridge Over the River Usk, at Newport, Monmouthshire".

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. D. Reid: "Mice and Men in Medical Research" (Thirty-fourth Stephen Paget Memorial Lecture), followed by the Annual General Meeting.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King: "Energy". (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on October 20, 26 and 27.)

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. C. B. Alcock: "Problems with Solutions: a Chemical View of High Temperature Materials" (Inaugural Lecture).*

SOCIETY OF CHEMICAL INDUSTRY (joint meeting of the Heavy Organic Chemicals Group and the Plastics and Polymer Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. H. Briston: "The Use of Chemical Products in Packaging".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Dr. C. Kendrick: "Quality Control in the Mass Production of Plastics Mouldings".

Wednesday, October 20

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. B. W. Meade: "Laboratory Aspects of Drug Overdose".*

INSTITUTION OF CHEMICAL ENGINEERS (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Dr. A. J. V. Underwood, O.B.E.: "Chemical Engineering—Reflections and Recollections".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Computer/Instrument Interfaces".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. R. Carstairs: "Six Years' Operation of Cyclone Boilers at Thornton Power Station".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7) at 6 p.m.—Mr. D. J. Lewis and Mr. A. S. Evans: "Radio Frequency Interference Control".

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. P. Fink-Jensen (Denmark): "Rheological Properties of Paint and Two New Instruments for Measuring Them" (European Co-operation Lecture).

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 7 p.m.—Prof. N. S. Sutherland: "Attention and Learning in Animals".*

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the South-East Essex Technical College and School of Art Chemical Society, at Longbridge Road, Dagenham, Essex), at 7.30 p.m.—Meeting on "Computer Applications in Chemistry".

Thursday, October 21

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. M. J. Brown: "Do We Need Cities?"*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. Patric Dickinson: "Computer versus Creator".*

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, Imperial College, London, S.W.7), at 2 p.m.—Symposium on "Organic Chemical Approaches to Biosynthesis".

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Management Training for Engineers" introduced by Mr. D. L. Marples.

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, London, W.C.1), at 5.30 p.m.—Sir George Godber: "Scientific Progress and the Pattern of Medical Care". (First of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. E. Woodcock: "Cooling Coil Evaluation".

SOCIETY FOR ANALYTICAL CHEMISTRY, Biological Methods Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Use and Abuse of Statistics in Biological Assays" opened by Mr. K. L. Smith.

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Prof. G. Macdonald: "On the Scientific Basis of Tropical Hygiene" (Presidential Address).

UNIVERSITY OF LONDON (at Wye College, Wye, Ashford, Kent), at 8.15 p.m.—The Rt. Hon. Lord Northbourne: "Religion and Science".*

Thursday, October 21—Friday, October 22

POWDER METALLURGY JOINT GROUP OF THE IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS, in association with the BRITISH METAL SINTERINGS ASSOCIATION (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 11 a.m. on Thursday and 10 a.m. on Friday—Annual Meeting.

Friday, October 22

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. B. D. Shaw: "Explosives".

Saturday, October 23

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Poul Rovsing Olsen (Copenhagen): "The Greenland Eskimos and Their Music".*

Monday, October 25

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Symposium on "The Changing Face of Reinforced Plastics".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. E. B. Atkinson: "Flow Behaviour of Molten Polymers".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. E. G. Bowen: "The Welsh Colony in Patagonia".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. F. W. Gibbs: "Gunpowder Priestley and Dictionary Johnson".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Sir John Cockcroft, O.M., F.R.S.: "Competitive Nuclear Power".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.–I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. W. J. Poppelbaum: "Opto-Electronics".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. J. T. Bull: "A.C. Traction Power Supplies" (Chairman's Address).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER or LECTURER (preferably with a special interest in analysis) IN PURE MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (October 20).

ASSISTANT LECTURER or COLLEGE LECTURER (with an honours degree in agricultural science, chemistry or biochemistry with suitable postgraduate experience) IN AGRICULTURAL CHEMISTRY—The Secretary and Bursar, University College, Dublin, Republic of Ireland (October 22).

ASSISTANT LECTURER (with a good honours degree or equivalent and preferably some research or industrial experience) IN BOTANY; and an ASSISTANT LECTURER (with a good honours degree or equivalent and preferably some research or industrial experience) IN ZOOLOGY—The Academic Registrar, Brunel College, Woodlands Avenue, London, W.3 (October 22).

COLLEGE LECTURER IN GENETICS IN THE AGRICULTURAL BIOLOGY DEPARTMENT, FACULTY OF GENERAL AGRICULTURE—The Secretary and Bursar, University College, Dublin, Republic of Ireland (October 22).

SENIOR LECTURER IN MATHEMATICS—The Registrar, Loughborough Training College, Loughborough, Leicestershire (October 22).

COMPUTER OFFICER (with a suitable university degree and experience in computer programming and operation) AT THE TIDAL INSTITUTE AND OBSERVATORY—The Registrar, The University, Liverpool, quoting Ref. CV/296/N (October 23).

KEEPER (with a good honours degree in botany and/or the Museums Association Diploma; an interest in economic botany, museum experience of fieldwork, and preferably a working knowledge of at least one modern foreign language) OF BOTANY—The Director, City of Liverpool Museums, William Brown Street, Liverpool, 3 (October 23).

PHYSICS LABORATORY TECHNICIAN FOR A GROUP ISOTOPE LABORATORY—The House Governor (A), Charing Cross Hospital, London, W.C.2 (October 23).

CHIEF STOREKEEPER (with experience in the supply and maintenance of scientific or technical laboratories) IN THE DEPARTMENT OF CHEMISTRY—Prof. Stanley Peat, Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire, North Wales (October 25).

LECTURERS IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne 2 (October 27).

CHAIR OF THERMODYNAMICS AND FLUID MECHANICS IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast, Northern Ireland (October 30).

LECTURER (registered medical practitioner holding the D.P.M., with broad experience of different aspects of psychiatry) IN PSYCHIATRY—The Registrar, The University, Leeds, 2 (October 30).

SENIOR BIOCHEMIST to take charge of a routine CHEMICAL PATHOLOGY DEPARTMENT—The Secretary, Queen Mary's Hospital, Roehampton, London, S.W.15 (October 30).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 31).

RESEARCH FELLOW (with an honours degree in a relevant subject and a minimum of two years postgraduate experience or training) IN RADAR AND SIGNAL PROCESSING IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham, 15 (November 1).

LECTURER IN EDUCATION, and a LECTURER IN SOCIOLOGY/SOCIAL ANTHROPOLOGY AT THE UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND—The Secretary, Inter-University Council for Higher Education Overseas, 33, Bedford Place, London, W.C.1 (November 2).

MYCOLOGIST (with an honours university degree in science or equivalent, and preferably suitable research experience) IN THE SOIL BIOLOGY DEPARTMENT—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (November 4).

SENIOR DEMONSTRATOR (with a degree in psychology and preferably some research or teaching or professional experience) IN PSYCHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, November 5).

SENIOR LECTURER or LECTURER (with an honours degree in zoology) IN ZOOLOGY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 5).

CHAIR OF FLUID MECHANICS AND HYDRAULIC ENGINEERING at Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 12).

HEAD OF THE DEPARTMENT OF MATHEMATICS, South Australian Institute of Technology—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, South Australian Institute of Technology, North Terrace, Adelaide, South Australia (Australia, November 12).

CHAIR OF PHYSICS at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 15).

LECTURER or SENIOR LECTURER in the School of Botany, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 15).

LECTURERS (with a good honours degree and a keen research interest in any branch of electronics and control engineering) IN THE ELECTRONICS AND CONTROL DIVISION, School of Engineering Science—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

MASTER TO TEACH GEOGRAPHY AND MATHEMATICS—The Master, Dulwich College, London, S.E.21.

MASTERS TO TEACH (*a*) PHYSICS AND (*b*) MATHEMATICS (sixth form work)—The Headmaster, Forest School, Snaresbrook, London, E.17.

POSTDOCTORAL FELLOW (preferably with some experience in X-ray crystallography) IN THE DEPARTMENT OF CHEMISTRY, to collaborate with Dr. B. Stevens in a study of the photochemistry and radiation chemistry of crystalline aromatic compounds—The Registrar, The University, Sheffield.

POSTDOCTORAL RESEARCH BIOCHEMIST to join a group engaged in studying the control of protein biosynthesis in animal cells—Prof. H. R. V. Arnstein, Department of Biochemistry, University of London, King's College, Strand, London, W.C.2.

PROFESSOR IN PHYSICAL CHEMISTRY—Prof. Harry E. Gunnung, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (Academic) IN THE DEPARTMENT OF PHYSICS to assist in an investigation into laser-induced surface damage under the direction of Prof. S. Tolansky, F.R.S.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Design Method Symposium, University of Aston in Birmingham Designate (College of Advanced Technology), September 21, 22, and 23, 1965—Preprints. (Organized by the Design and Innovation Group.) Pp. 322. (London: Butterworths, 1965.) [149

Jacksons' Millboard and Fibre Co., Ltd. Directors Report and Accounts, 1965. Pp. 12. (London: Jacksons' Millboard and Fibre Co., Ltd., 1965.) [149

Royal Observatory Bulletins. No. 97: The Absolute Magnitudes of RR Lyrae Variable Stars. By Sir Richard Woolley, G. A. Harding, Anneila I. Cassells and Jennifer Saunders. Pp. E1-E30. 4*s*. 6*d*. net. No. 98: Proper Motions and Radial Velocities of Hyades Stars. By P. A. Wayman, L. S. T. Symms and K. C. Blackwell. Pp. E31-E62. 4*s*. 6*d*. net. (London: H.M. Stationery Office, 1965.) [149

The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom (excluding part-time and external degree courses). Third edition. Pp. 219. (London: The Association of Commonwealth Universities, 1965.) 11*s*. 6*d*. [149

University of Reading. National Institute for Research in Dairying—Report 1964. Pp. 164. (Shinfield, Berkshire: National Institute for Research in Dairying, 1965.) 7*s*. 6*d*. [149

Anti-Locust Bulletin No. 42: The Abdomen of the Female Desert Locust (*Schistocerca gregaria* Forskal), with special reference to the Sense Organs. By Joan G. Thomas. Pp. 20+45 figures. (London: Anti-Locust Research Centre, 1965.) 8*s*. 6*d*. [149

City of Liverpool College of Technology. Prospectus of Full-Time Courses, Session 1965–1966. Pp. 67. (Liverpool: City of Liverpool College of Technology, 1965.) [149

Road Research Laboratory: Ministry of Transport. Road Research Technical Paper No. 74: Traffic Signals for High-Speed Roads. By Dr. F. V. Webster and P. B. Ellson. Pp. v+49+2 plates. (London: H.M. Stationery Office, 1965.) 4*s*. 6*d*. net. [149

Other Countries

Conseil National de la Politique Scientifique, Bruxelles. Recherche et Croissance Économique: Rapport sur Certains Aspects de la Recherche Industrielle dans ses Relations avec la Croissance Économique. Pp. 218. (Bruxelles: Conseil National de la Politique Scientifique, 1965.) [149

World Health Organization. Public Health Papers, No. 27: Trends in the Study of Morbidity and Mortality. By Dario Curiel and 13 other authors. Pp. 196. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 8 Sw. francs; 13*s*. 4*d*.; 2.75 dollars. [149

Council on Research in Bibliography, Inc. Mental Health Book Review Index, Vol. 10, 1965. Compiled by the Editorial Committee and Contributing Librarians. Pp. xx+78. Annual subscription: 5 dollars. (New York: Council on Research in Bibliography, Inc., c/o Research Centre for Mental Health, New York University, 1965. Subscriptions etc. to Miss Lois Afflerbach, Paul Klapper Library, Queens College, Flushing, New York, 11367.) [149

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Index to Reports of Geological Survey of Canada from 1951–59. Compiled by J. F. Wright. Pp. xii+379. (Ottawa: Queen's Printer, 1965.) 10 dollars. [149

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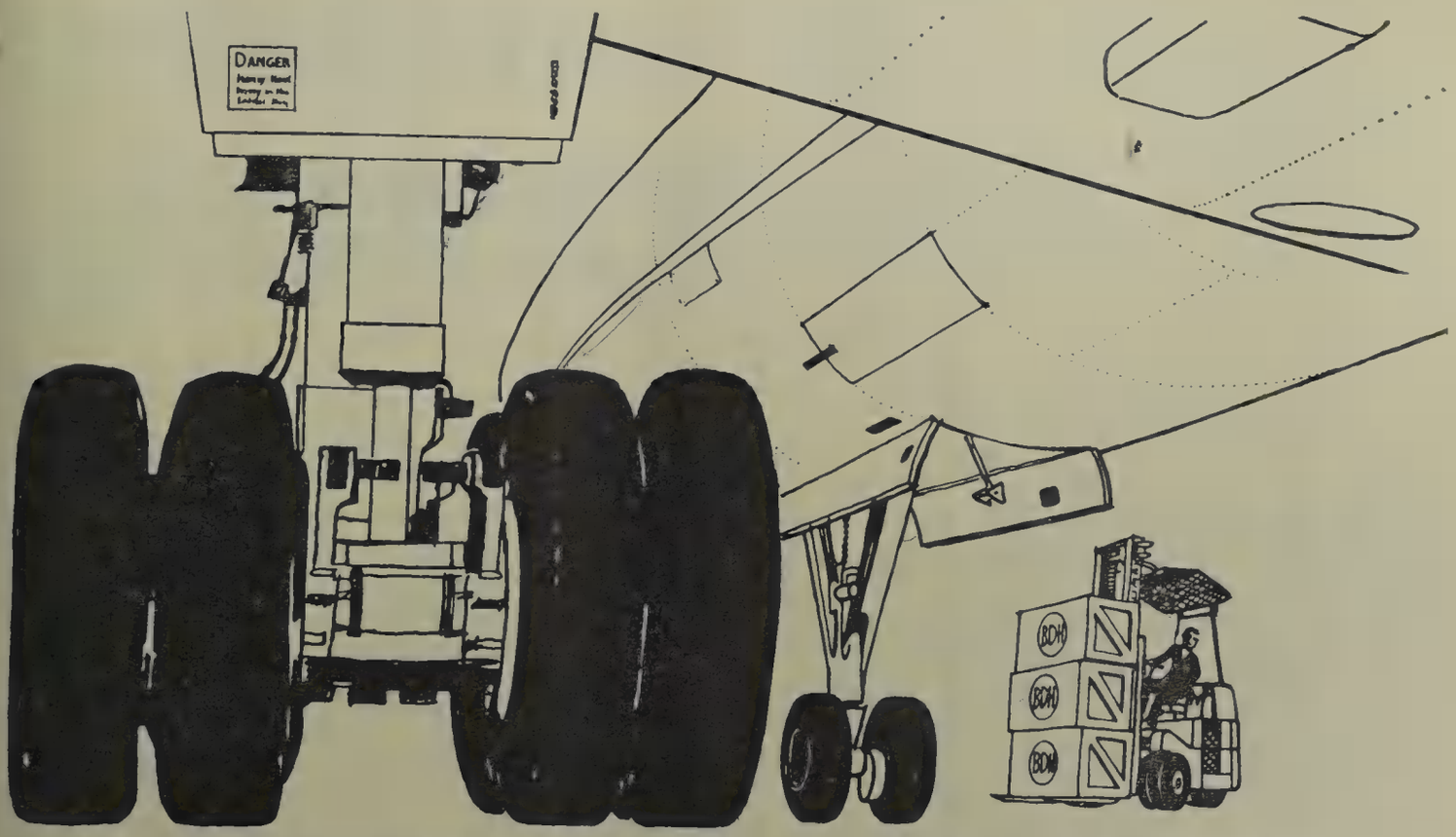

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The Marine Department require a Director of Research for the Fisheries Research Division, Wellington. The initial salary will be £2,900 or £3,100 per annum.

Fisheries research, carried out by the New Zealand Government, has been reorganized as a separate division of the Marine Department, with emphasis on basic fisheries research and separate from the development, extension, management, and technology functions, which are the responsibility of an associated Division of the Department. An excellent opportunity exists for a capable and experienced fishery biologist, with a capacity for administration and leadership, to develop a new unit under very favourable circumstances, with Government priorities being given to research expansion, provision of new laboratories, research vessels and associated facilities. There has been some basic work carried out in New Zealand on demersal species and on invertebrates; however the present and continuing need is for substantial expansion of the basic biological studies on which valid management practices can be founded. Priority is being given to building up a fully qualified graduate staff, and a technical staff. The Director's immediate responsibilities will include the recruitment of staff, formulation of a research programme, and the planning of a laboratory and ship facilities.

The initial salary will be £2,900 or £3,100 but there are prospects of substantially higher remuneration in the expansion scheme and on individual merit. Conditions of appointment include provision for payment of fares and other travelling and accommodation expenses. There is opportunity to join an advantageous superannuation scheme.

While it is hoped to make a permanent appointment, a contract for approximately five years, with appropriate travel and superannuation arrangements might be available for a suitable candidate unable to take up a permanent position.

Further details are available from: The Secretary, Marine Department, P.O. Box 2395, Wellington (for attention Fisheries Research Division).

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For Grade III posts candidates must, in addition, normally have had at least three years' further study or research and/or employment providing experience of equivalent value, and, for posts in horticulture, experience in commercial work. For Grade IV (a) posts first- or second-class honours degree or equivalent or higher qualification essential.

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Excellent conditions of service include a contributory pension scheme and free life assurance. The laboratory is pleasantly situated 11 miles South of Cambridge, and free transport is provided to and from Saffron Walden, Newport and Cambridge, each day.

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A qualified Statistician, who may have graduated recently is required for a diverse range of work at the Authority's statistical unit at Letchworth, Hertfordshire. The work includes the design and analysis of experiments and surveys, and population genetics, and offers scope for initiative and the development of particular interests. Analysis of data, including that from pig testing schemes, will be aided by the installation shortly of an NCR-Elliott 4100 computer. The salary scale for this appointment is £1,050 to £1,715 per annum.

Further details and application forms may be obtained from the Establishment Officer, Pig Industry Development Authority, 15 Ridgmount Street, London, W.C.1, to whom completed forms should be returned by November 1, 1965.

APPLICATIONS ARE INVITED FOR THE position of Head of Department and Staff positions in the Department of Chemistry and Chemical Technology in the West Kootenay Regional College, Castlegar, B.C., Canada. The College will open in September, 1966, to some 400 students and will stress excellence in teaching and will encourage an experimental approach to the curriculum.

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Starting salaries of up to $10,000 per annum are offered depending on qualifications and experience. Excellent pension plan and other benefits. Travel and removal grants available.

Please send curriculum vitae and the names of three referees to: The Principal, West Kootenay Regional College, 1385 Cedar Avenue, Trail, British Columbia, Canada.

UNIVERSITY OF TASMANIA
LECTURER IN ZOOLOGY

The University invites applications for the appointment as Lecturer in the Department of Zoology. The salary scales for lecturers are Grade II: £A2,400 by £A100 to £A2,700 and Grade I: £A2,800 by £A100 to £A3,200. An appointment will be offered within either grade according to qualifications and experience. Research work currently being conducted in the Department includes parasitology, insect physiology, marine biology and marsupial ecology. The professor-elect of Zoology is Dr. Bruce Johnson, and he will take up his appointment in January, 1966. Dr. Johnson's research interests are in neuroendocrinology and electron microscope studies of neurosecretion. The University will particularly welcome applications from those interested in comparative physiology or animal behaviour but those with other interests are also invited to apply.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 10, 1965.

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If you would like an appointment with them, would you write as soon as possible and preferably before 7th December, 1965 for East Coast Candidates to:—

Dr. J. A. Saxton, Director,
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AUSTRALIA

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The Division of Mathematical Statistics has its headquarters at the University of Adelaide, S.A. and officers of the Division are also stationed at key points throughout the various establishments of the Organization to provide specialized assistance in planning research and in analysis of experimental results. The Division also undertakes fundamental research in various branches of mathematical statistics.

An appointee, depending upon his particular qualifications, will be required to take part in the Divisional research programme being conducted at one of the centres located in Townsville (Pastoral Research Laboratory), Sydney (National Standards Laboratory), Melbourne (Division of Protein Chemistry) or Adelaide (Divisional Headquarters).

Applicants should have a Ph.D. degree in Arts or Science with Mathematics and Mathematical Statistics as major subjects, or have had postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability.

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Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of a fixed-term appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.,

to whom applications (quoting Appointment No: 440/182) should be addressed by November 13, 1965.

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Applications should be sent not later than October 20, 1965, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained on quoting reference 174/65/Na.

UNIVERSITY OF ILLINOIS

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CHICAGO, ILLINOIS 60612

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Applications should reach Dr. Ralph F. Voigt, Head of Department of Pharmacognosy and Pharmacology by November 15, 1965.

The proposed

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LECTURER OR ASSISTANT LECTURER IN OPERATIONAL RESEARCH

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Further details and application form from Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7. Please quote reference number CST 65/105.

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Further particulars and forms of application from the Staff Officer, College of Advanced Technology, Gosta Green, Birmingham, 4, to whom applications should be forwarded within fourteen days of this advertisement. Please quote 434/6.

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QUALIFICATIONS: normally first- or second-class honours degree, Diploma of Technology or an equivalent or higher qualification in a scientific or technological subject. Candidates should also have a keen interest in economic development through innovation and technological change and in the industrial application of research, together with industrial experience, mobility, initiative, and an aptitude for dealing with people.

SALARY (National): £2,174 to £3,002. Starting salary may be above minimum. Promotion prospects.

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(ii) MATHEMATICIAN with interest in formulation of mathematical models, to interpret and describe kinetics of events which occur in rivers, estuaries, and coastal waters, and in biological treatment processes; and to advise and assist staff in mathematical aspects of their work. Experience in use of electronic computers and in statistical analysis of data an advantage. Knowledge of theories of molecular and convective diffusion and of turbulent mixing of fluids useful. (Ref.: E/AM/019.)

(iii) MICROBIOLOGIST, with special interest in kinetics of growth of micro-organisms, to initiate research in population dynamics of mixed cultures formed in biological treatment plants, in order to interpret observed changes in activity and state of aggregation of the biomass with changes in operating conditions and to study self-purification in rivers and the survival of organisms in effluents discharged into the sea. (Ref.: E/AM/020.)

(iv) CHEMICAL ENGINEER, PHYSICAL CHEMIST or PHYSICIST for fundamental studies on rivers and estuaries including especially work on mass and heat transfer at the air-water interface, mixing and dispersion, diffusion in sediments and stagnant water and on kinetics of biological oxidation processes. Initially this involves investigating factors governing rates of mass-transfer of atmospheric oxygen into water flowing in a model channel. Interest in hydraulics and hydrodynamics an advantage. (Ref.: E/AM/029.)

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UNIVERSITY OF GLASGOW

DEPARTMENT OF PSYCHOLOGICAL MEDICINE

PSYCHOSOMATIC RESEARCH UNIT

BIOCHEMIST

Applications are invited for the above research appointment. Candidates should have an honours degree in Biochemistry and have had some research experience or possess a Ph.D. The Biochemist is required to assist with the investigation of aspects of endocrine function in certain bodily illnesses in human subjects. He will have the support of the University Department of Steroid Biochemistry. A well-equipped laboratory and technical assistance is available. Salary on Whitley Council Scale for Biochemists with placement according to qualifications and experience.

Further details can be obtained from the Director of the Psychosomatic Research Unit, Southern General Hospital, Glasgow, S.W.1, to whom applications, stating age, qualifications, experience and the names of two referees, should be sent by November 5, 1965.

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF CIVIL ENGINEERING

ESTUARINE AND COASTAL HYDROGRAPHY

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Applications with full details of qualifications should be addressed to Professor W. Frazer, Head of Civil Engineering Department, Montrose Street, Glasgow, C.1, not later than Friday, October 22, 1965.

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SCIENTIFIC OFFICER

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Applications to The Director, Tropical Pesticides Headquarters and Information Unit, Tropical Products Institute, 56/62 Grays Inn Road, London, W.C.1, by October 22, 1965.

NATIONAL UNIVERSITY OF IRELAND

Research Assistant required for new multidisciplinary laboratory in cancer or vascular field, biochemistry or tissue-culture background, experience with general biological experimentation an asset. Salary range £700 to £1,000.

Applications to Professor S. M. Lavelle, Department of Experimental Medicine, University College, Galway.

Electron Microscopy and Diffraction Techniques

Applications are invited from Senior Scientists experienced in the application of Electron Microscopy and Electron Diffraction Techniques to the study of the structure of solids.

Electron microscopy is being increasingly used to further our research on the role of both lattice defects in crystals and micro structure in general, as well as on the physics and chemical properties of materials in bulk and thin film form.

Experience of dislocation physics, the strength of solids and diffraction theory would be an advantage.

This is a senior appointment within the Research Department of the company. A realistic salary will be paid and applications from scientists living abroad are welcome.

Apply, in confidence, to the Staff Manager, MORGANITE RESEARCH AND DEVELOPMENT LIMITED, Battersea Church Road, London, S.W.11.

UNIVERSITY OF DUBLIN

Trinity College

SCHOOL OF VETERINARY MEDICINE

LECTURER IN VETERINARY MEDICINE

Applications are invited for the above post. A veterinary qualification is essential. Salary in the range £1,500 by £50 to £2,100 plus marriage and child allowances and all pension provision paid by the College. The successful candidate will be placed on the scale at a point appropriate to his experience and qualifications.

Further information may be obtained from the Secretary, Trinity College, Dublin, 2, who will receive applications until November 15, 1965.

UNIVERSITY OF LONDON KING'S COLLEGE

LECTURER IN BIOCHEMISTRY

The College will require on or after January 1, 1966, a Lecturer in Biochemistry.

Salary scale: £1,805 rising by increments of £100 to £2,505 plus £60 London Allowance.

Conditions of appointment and application forms may be obtained from the Registrar, King's College, Strand, London, W.C.2; completed forms must reach him by October 31.

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a Post-doctoral Research Biochemist, to join group engaged in studying the control of protein Biosynthesis in animal cells. Salary within the range of £1,400 to £1,600.

Applications to Professor H. R. V. Arnstein, Department of Biochemistry, University of London, King's College, Strand, London, W.C.2.

UNIVERSITY OF SINGAPORE

Applications are invited for (a) Lectureship or (b) Assistant Lectureship in Geography. Salary scales: (a) £1,148 to £1,820 per annum; (b) £1,001 to £1,099 per annum. Expatriation and cost of living allowances. Family passages. Subsidized accommodation. Provident Fund Scheme.

Detailed applications (eight copies), naming three referees by November 15, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

RESEARCH OFFICER (VIROLOGIST)

EAST AFRICAN COMMON SERVICES ORGANISATION

Required to study virus diseases affecting agricultural crops and to help with the Scientific Supervision of the East African Plant Quarantine Station.

Candidates must hold an honours degree in botany or agricultural botany, with experience in plant virology.

Salary scale £1,221 to £2,097 a year plus 25 per cent terminal gratuity. A 12½ per cent increase in salary has been approved. Quarters available. Passages provided. Two-year contract. Education allowances. Generous leave.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/214/011 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

AGRICULTURAL CHEMIST

UGANDA

Required for research work on soil fertility problems connected with tropical rotations, manures and fertilizers.

Candidates must hold a second-class honours degree in agricultural chemistry or chemistry, plus at least two years' post-graduate training or experience in soil science.

Salary scale £1,374 to £2,757 a year plus 25 per cent terminal gratuity.

Passages provided. Generous leave. Government quarters. Education allowances.

Appointment on contract for one tour of 21 to 27 months.

Applicants, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of career and qualifications, quoting RC. 213/183/022 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

CITY OF LIVERPOOL MUSEUMS

Applications are invited for appointment as Keeper of Botany. Salary £1,610 to £1,940 (Grade B). Qualifications must include a good honours degree in botany and/or the Museums Association Diploma; an interest in Economic Botany, and Museum experience of fieldwork. A working knowledge of at least one modern foreign language is also desirable.

Further particulars from the Director, City of Liverpool Museums, William Brown Street, Liverpool, 3. Closing date October 23, 1965.

THOMAS ALKER,
Town Clerk.

SK&F

have a vacancy for a

SCIENTIFIC OFFICER

in their

BIOCHEMISTRY DEPARTMENT

Smith Kline & French Laboratories Limited is a leading pharmaceutical Company with an international programme of basic research and product development. The recently formed Biochemistry Department will shortly be housed in new laboratories.

There is now a vacancy for a Scientific Officer who will primarily be responsible for the clinical biochemistry carried out in animals undergoing toxicity tests. He will also participate in one of the research projects either on the drug metabolism side or on a biochemical research topic.

The successful applicant will have received at least part of his training in a hospital laboratory, will probably have F.I.M.L.T. or H.N.C. as a minimum qualification, and preferably now be employed in the drug industry and have had some experience in drug metabolism or biochemical research. It is unlikely that he will be less than 27 years old.

The Company has a healthy and progressive salary structure, a non-contributory Pensions and Life Assurance Scheme and can, in certain circumstances, help married men with housing and removal expenses.

Please write, in confidence, giving age, present salary, qualifications, and any practical/theoretical experience, to :

The Personnel Officer,
SMITH KLINE & FRENCH LABORATORIES LIMITED,
Mundells, Welwyn Garden City, Herts.
quoting Reference No. 06/70/30.

SUNDERLAND EDUCATION AUTHORITY

SUNDERLAND TECHNICAL COLLEGE

Department of Chemistry and Biology

RESEARCH POSTS

Applications are invited for immediate appointments to the following posts:

(1) Research Assistant, to assist in the development of a research project in physical chemistry. Candidates should possess a degree, H.N.C., or H.N.D. in Chemistry and preferably have had some experience in research and/or industry.
Salary: £800 by £50 to £1,000 per annum.

(2) Research Studentships, to work in the following fields:
(a) The erosion of oxide films on metals
(b) The preparation and relative acidity of substituted diphenylamines.

Candidates should be graduates (or equivalent) in Chemistry. The Studentships are of annual value £500, which may be supplemented by paid part-time teaching. Successful candidates are normally expected to submit the results of the work for a higher degree.

Further particulars and application forms for the above positions may be obtained from the Registrar, The Technical College, Sunderland, Co. Durham. Completed applications should be returned to the Director of Education, 15 John Street, Sunderland by October 30, 1965.

If you are a scientist can you communicate?

The post: Science Talks Producer, Talks and Current Affairs Department, BBC Radio. Responsible to Editor, Science Talks for the production of talks and discussion programmes reflecting the place of science and technology in the contemporary scene. Will involve creating programme ideas, briefing contributors, some editorial work on scripts, and rehearsal and production in the studio.

The requirement: University degree in science; familiarity with current advances in scientific thought; ability to communicate science effectively on radio.

The salary: appointment may be made either on a scale £1,950 rising by annual increments of £115 to £2,525, or a scale £1,570 by annual increments of £90 and £95 to £2,270.

Write for application form (enclosing addressed envelope and quoting reference 65.G.620.N.) to Appointments Department, BBC, London, W.1, within five days.

BBC RADIO

INSPECTOR GRADE III
(SCIENTIFIC OFFICER)

required in the Departments of Lands, Dublin, for work in connection with the development of game resources. Salary scale, £805 to £1,825 entry up to £1,319 depending on qualifications and experience. Maximum age limit: 35 years. Essential: a recognized first- or second-class honours University degree in which Zoology was taken as a major subject.

Apply, preferably by postcard, to Secretary (S.18), Civil Service Commission, 45 Upper O'Connell Street, Dublin, 1. Latest time for receiving completed application forms: 5.30 p.m. on October 26, 1965.

UNIVERSITY OF TASMANIA

LECTURESHIP IN ELECTRICAL ENGINEERING

The University invites applications for the position of Lecturer in Electrical Engineering. The salary scales for lecturers are Grade II: £A2,400 by £A100 to £A2,700 and Grade I: £A2,800 by £A100 to £A3,200. An appointment will be offered within either grade according to qualifications and experience. Applicants should hold an honours degree in electrical engineering and should give details of their particular fields of interest.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 10, 1965.

UNIVERSITY OF LEEDS

DEPARTMENT OF AGRICULTURE
RESEARCH ASSISTANT IN PLANT PATHOLOGY

Applications are invited from graduates in Botany or Agricultural Botany for a Research Assistantship to work on the formation of cucumber stem lesions by *Mycosphaerella citrullina* and *Botrytis cinerea*. Applicants should have an interest in plant pathology. The appointment is for one year, with the possibility of renewal for a further two years. Salary £600 per annum plus superannuation.

Applications (three copies), stating age, qualifications and experience, together with the names of three referees, should reach the Registrar, the University, Leeds 2 (from whom further particulars may be obtained), not later than October 25, 1965.

Geigy *The discovery and development of a number of outstandingly successful pharmaceutical preparations used in the treatment of rheumatic and nervous disorders have given Geigy an international reputation in this field.*

RESEARCH CHEMIST

There is a vacancy for a Research Chemist to work on the synthesis of compounds of medicinal interest. The successful candidate will probably be aged 25 to 30 years and will hold a good Honours degree and preferably a higher degree in chemistry. He should already have several years' experience in synthetic organic chemistry, which may have been obtained in industry or at an educational institution.

The appointment will be in one of the company's pharmaceutical research laboratories which are situated in a modern, well-equipped laboratory building at one of the company's main locations in Trafford Park, near Manchester.

Geigy (U.K.) Ltd. is a member of a large international group of companies with world-wide interests in the field of fine chemicals and pharmaceuticals. The organisation adopts a very progressive attitude towards the introduction of new techniques and ideas in both management and research and development.

A good competitive salary will be paid, contributory pension scheme and other conditions in keeping with modern practice.

Please write, quoting Ref. G.25/BO, giving brief details of experience and qualifications, to:

Personnel Officer,
Geigy (U.K.) Ltd.,
Simonsway, Manchester, 22.

WEST HAM COLLEGE OF TECHNOLOGY

(A Regional College and an Institution of the University of London)

DEPARTMENT OF MATHEMATICS AND STATISTICS

A vacancy exists for

A PRINCIPAL LECTURER

to teach Mathematics to full-time students reading for internal degrees of the University of London, including B.Sc. Special Mathematics.

Applicants should possess a higher degree and have had appropriate teaching, industrial or research experience and should have a special interest in Pure Mathematics and/or Statistics. The person appointed will be expected to take an active part in the development of postgraduate and specialist courses within the department and to give some assistance with administration.

Further particulars of this appointment and forms of application may be obtained from the Vice-Principal, West Ham College of Technology, Romford Road, Stratford, London, E.15, to whom forms should be returned by October 22, 1965.

FISONS PHARMACEUTICALS LIMITED

CHIEF PATHOLOGIST

FOR THERAPEUTIC RESEARCH

FISONS PHARMACEUTICALS LIMITED, wish to appoint a graduate in veterinary science or medicine as Chief Pathologist of its Research Laboratories. The successful candidate will be responsible to the Research Director for the Histology and Chemical Pathology Sections. The work will entail an evaluation of the toxicity of pharmaceutical compounds on a wide range of laboratory animals and will involve close co-operation with the Chief Toxicologist and senior members of staff. The post is suitable for a young graduate, with a few years' experience in a similar field, who has an experimental approach to Pathology and now merits greater responsibility.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications, which will be confidential, should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

UNIVERSITY OF TASMANIA

LECTURER IN PHYSICS

The University invites applications for the position of Lecturer in Physics. The salary scales for Lecturers are Grade II, £A2,400 by £A100 to £A2,700 and Grade I, £A2,800 by £A100 to £A3,200. Appointment will be offered within either grade according to qualifications and experience. Preference will be given to an applicant whose research interests are in astrophysics, optical astronomy, radio astronomy or plasma physics. Current research in the Department includes radio, optical and cosmic ray astronomy, theoretical plasma physics, biophysics and geometrical optics. Facilities include an Elliot 503 computer.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 19, 1965.

SOIL SURVEY OFFICER

UGANDA

Required to carry out soil surveys as a member of a land planning team working on the development of group farms.

Candidates must hold an honours degree in either natural science, agriculture or geography, with, if possible, postgraduate training or experience in soil science.

Salary scale £1,374 to £2,757 a year, plus 25 per cent terminal gratuity. Passages provided. Education allowances. Government quarters. 21 to 27 months contract. Generous leave.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC. 213/183/019 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

SOIL CONSERVATION ENGINEER

UGANDA

Required to carry out soil conservation measures on projects, to check layouts, to train field staff and to collect data and prepare a handbook.

Candidates must hold a second-class degree in agricultural engineering, or a degree in soil conservation, or a degree in civil engineering and soil conservation. Experience in soil conservation is desirable.

Salary scale £1,374 to £2,757 a year plus 25 per cent terminal gratuity. Government quarters. Education allowances. Passages provided. 21 to 27 months contract. Generous leave.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/183/020 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

BIOLOGIST, PREFERABLY WITH PH.D. or medically qualified, for a research post in a unit working on trophoblastic tumours and leukaemia in the Charing Cross Hospital Group. Applicants should have interests or experience in the problem of graft versus host reaction, immunological tolerance and mitotic synchronization. Initial salary up to £1,800 per annum, depending on qualifications and experience.—Applications to: Dr. K. D. Bagshawe, Edgar Laboratory, Fulham Hospital, London, W.6.

SENIOR SCIENTIST

MORGANITE RESEARCH AND DEVELOPMENT LIMITED
Battersea Church Road, London, S.W.11

Morgan

A Senior Scientist, at least 28 years old, and with several years' post-graduate experience, is required to lead a research group at our Northfields Laboratories, Wandsworth. The group will study the mechanical properties and strength of ceramics in relation to structure, composition and method of fabrication.

Experience in some or all of the following fields is essential : creep, fracture, deformation studies over a wide temperature range, structure of solids, dislocation physics and techniques for studying lattice defects in crystalline solids.

This is a senior appointment with interesting possibilities for a scientist who enjoys initiating research work. If you are interested in this field or in other areas of materials science, please write to the Staff Manager, quoting reference NQg2, giving details of your qualifications and experience or, if you prefer, ask for an application form to be sent to you for completion. Applications from scientists working abroad will be welcome.

UNIVERSITY OF OTAGO MEDICAL SCHOOL

DUNEDIN, NEW ZEALAND

LECTURER OR SENIOR LECTURER IN CHEMICAL PATHOLOGY

Applications are invited for the position of Lecturer or Senior Lecturer in Chemical Pathology. Salary according to experience and qualifications, within the following scales: Science graduate, £1,300 by £100 to £2,000 to £2,500; Medical graduate, £2,000 by £100 to £2,500 to £3,000. The status of Senior Lecturer may be awarded as a mark of personal distinction either on initial appointment or by subsequent promotion. The salary of a senior lecturer is not less than £2,300 for a science graduate and £2,700 for a medical graduate. Duties include teaching and participation in diagnostic services. The Department has an excellent technological staff. Research is encouraged and the appointee would have opportunity to study for a higher degree. Family passages and assistance with removal costs are provided. The appointee is expected to commence duties in March, 1966, or as soon thereafter as possible.

Further particulars are available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or from the Registrar of the University. Applications, with the names of three persons to whom reference can be made, close in New Zealand and London on December 15, 1965.

UNIVERSITY OF MALAYA

DEPARTMENT OF PHYSICS

Applications are invited for appointment as Lecturer/Assistant Lecturer in the Department of Physics. Candidates should have at least a good honours degree with suitable teaching and research experience. Preference will be given to applicants specialized in some branch of Solid State Physics.

Salary scale: The amounts mentioned below refer to Malayan dollars (per mensem) and their approximate sterling equivalents (per annum), are shown in parentheses. Lecturer: $920 by $40 to $1,160/$1,200 by $45 to $1,425 (£1,338 by £56 to £1,624/£1,680 by £63 to £1,995); Assistant Lecturer: $800 by $40 $880 (£1,120 by £56 to £1,232). In addition the following allowances are payable: Variable allowance: 35 per cent of basic salary, subject to certain maxima depending on marital status, minimum $150 per mensem (£210 per annum), maximum $400 per mensem (£560 per annum). Inducement allowance (if awarded), varies: (a) For Lecturers, from $0 to $300 per mensem (£420 per annum); (b) For Assistant Lecturers, from $0 to $200 per mensem (£280 per annum). Housing will be provided, if available, for which a rent of $100 to $125 per mensem (£117 to £175 per annum) will be charged. Provident Fund: Staff members contribute 5 per cent and University 15 per cent of basic salary.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. The closing date for the receipt of applications in Kuala Lumpur and London is November 15, 1965.

UNIVERSITY OF AUCKLAND, New Zealand

Applications are invited for the following appointments, the closing dates for which are shown in brackets :

LECTURESHIP IN PSYCHOLOGY : Applicants should possess postgraduate qualifications in Experimental Psychology and have research interests in the field of Visual Perception. The successful applicant would be expected, in co-operation with the Senior Lecturer in Optometry, to make a contribution from the psychological viewpoint, to the optometry course, particularly in connection with the functional organization of the visual system. (November 30.)

LECTURESHIP IN ENTOMOLOGY : Applicants must have qualifications in Entomology. Research experience in Ecology would be an added recommendation. (November 30.)

It is not necessarily a prerequisite for appointment as Lecturer that a candidate should already have held the position of assistant lecturer or its equivalent at another university institution.

Salary : Commencing salary within the appropriate scale will be determined in accordance with qualifications and experience. In the case of new members of staff coming from overseas, the current practice is to pay salaries from an approved sailing date.

Lectureship : £1,300 per annum, rising to £2,000 per annum. In normal circumstances a Lecturer whose services prove satisfactory to the Council may expect in due course to be given the status of a Senior Lecturer (salary maximum £2,500 per annum).

Travel and removal expenses allowed within specified limits. Where necessary provision is made for the continuation of the employer's contribution on unencumbered F.S.S.U. policies held by new members of staff.

Upon the completion of five years' satisfactory service return fares on an approved basis to country of origin may be granted to the person appointed and his dependent wife and children, provided that such fares are not being met from some other source.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

The Wellcome Research Laboratories

MEDICAL VIROLOGIST

Applications are invited for a post in the Department of Virology from medical graduates wishing to undertake, or continue with, a career in Virology. Previous experience of research and laboratory techniques would be an advantage although not essential, since there are very good facilities for training and acquiring experience. The successful applicant will have the prospect of a career in a large department with excellent laboratory facilities in which research and production programmes covering a wide field of human virology are being prosecuted. There is considerable scope for personal initiative, and publication of scientific papers is encouraged.

Applications, giving full personal particulars, should be sent quoting U. 163, to the Senior Personnel Officer, The Wellcome Research Laboratories, Langley Court, BECKENHAM, Kent, from whom further details may be obtained.

STATISTICIANS

27 PENSIONABLE posts in the London area for men or women aged at least 28.

QUALIFICATIONS: first- or second-class honours degree or Dip.Tech. in statistics, or other appropriate main subject combined with statistics. Other graduates with specially suitable statistical experience considered. Wide statistical experience essential.

SALARY (Inner London): £2,259 to £3,087. Starting salary may be above minimum. Promotion prospects.

WRITE to Civil Service Commission, Savile Row, London, W.1, for application form, quoting 6258/65. Closing date November 16, 1965.
(18)

CHEMICAL ABSTRACTS SERVICE

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Our continued growth has created full-time permanent positions for chemists in Columbus, Ohio

ALL LEVELS OF TRAINING

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London interviews can be arranged Foreign Language ability desirable

Write:

PERSONNEL DEPARTMENT
CHEMICAL ABSTRACTS SERVICE
THE OHIO STATE UNIVERSITY
COLUMBUS, OHIO 43210

ASSISTANT CONSERVATORS OF FORESTS

ZAMBIA

Applications are invited for the following appointments:

(a) District Forest Officer. Experience of Forest administration is preferable and the candidate appointed will be expected to supervise a district.

(b) Forest Survey Officer. Field experience in forest surveys and enumeration is necessary.

Candidates for both posts must have a degree in forestry.

Salary £1,180 to £2,600, depending on approved experience since graduation. Examples: With five years, starting point is £1,690 per annum; with ten years £2,300. In addition, a gratuity of 25 per cent of total emoluments received is payable on completion of contract. Education allowances are payable. Free air passages provided and quarters available. Three-year contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting Ref. RC 324/132/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

THE BRITISH DRUG HOUSES LTD.

APPOINTMENT OF BIOCHEMIST

A graduate is required for work in the Biochemical Development Department at Poole on the production of enzymes and other biochemicals.

Candidates should have an honours degree in Chemistry or Biochemistry, preferably with one or two years relevant research or industrial experience.

Applications, giving full details, should be addressed to the

PERSONNEL MANAGER,
B.D.H. LABORATORY CHEMICALS DIVISION,
POOLE, DORSET.

(RE-ADVERTISEMENT)

PROPOSED UNIVERSITY OF BRADFORD

(Bradford Institute of Technology)

DEPARTMENT OF PHYSICS

RESEARCH ASSISTANT OR RESEARCH STUDENT

Applications are invited for the appointment of a Research Assistant or Research Student (according to qualifications) to work in the Department of Physics with a small group investigating the application of existing and novel techniques of geophysical prospecting to archaeology. It is intended that the work shall lead to the award of a higher degree. Salary: Research Assistant £750 to £1,350 per annum; Research Student, £450 per annum.

Further particulars of the post and application form may be obtained from the Registrar, Ref. 126Y/X, Bradford Institute of Technology, Bradford 7.

Applicants who replied to the original advertisement are not required to submit a second application.

UNIVERSITY OF NEWCASTLE UPON TYNE

LECTURESHIPS IN ENGINEERING MATHEMATICS

The University invites applications for the post of Lecturer in Engineering Mathematics. This post, which has been newly-created, is for the current academic year and it is hoped that an appointment will be made as soon as possible. Two further Lectureships will be tenable from September 1, 1966. The commencing salary will be at an appropriate point on the Lecturers' scale £1,400 to £2,505. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, the University, Newcastle upon Tyne 2, with whom applications (three copies), together with the names and addresses of three persons to whom reference may be made, should be lodged within three weeks of the appearance of this advertisement.

MEDICAL RESEARCH COUNCIL'S METABOLIC Reactions Research Unit, Biochemistry Department, Imperial College, London, S.W.7, require a young biochemist mainly to participate in research on problems of intermediary metabolism connected with the mode of action of insulin. Salary, plus London allowance and superannuation will be dependent upon age, qualifications and experience.—Applications, giving full details and names of two professional referees should be sent to the Director at the above address.

THE SWINBURNE TECHNICAL COLLEGE

INSTRUCTOR IN ORGANIC CHEMISTRY

Applications are invited for appointment to this position to commence duties if possible in February, 1966.

Applicants should possess an Honours or higher degree and preferably had teaching and or industrial experience.

SALARY RANGE: Up to £2,575

Subject to certain conditions the salary for an Instructor can increase to £2,775 per annum.

Commencing salary according to qualifications and experience.

Superannuation, sick leave, and long service benefits are available, and assistance is given to appointees from overseas.

Applicants should supply the following information: Age, country of birth, marital status, educational qualifications, teaching, research or industrial experience, war service (if any), availability, copies of three recent testimonials and any other information thought relevant.

A schedule of duties and conditions of appointment can be supplied.

Applications should reach

The Director,
The Swinburne Technical College,
John Street, Hawthorne, Victoria, Australia.
by November 27, 1965.

LOTHIANS RIVER PURIFICATION BOARD

Applications are invited for the following post: Biologist. Salary grade £1,485 to £1,810. Applicants should possess: 1. An Honours Degree in Biology, preferably Zoology, with Chemistry or Microbiology as a subsidiary subject. 2. Associate Membership by examination of the Institute of Sewage Purification. Experience in Freshwater Ecology, Marine Biology and Oceanography relative to Pollution Prevention would be an advantage. The duties of the successful candidate will be the biological assessment of pollution in the rivers, burns and that part of the Forth Estuary within the Board's area. Post superannuable. Scottish J.I.C. conditions of service. Car allowance.

Applications, together with the names of two referees, to be submitted by November 3, 1965, to the Chief Technical Officer-River Inspector, Springwell House, 1 Georgie Road, Edinburgh, 11.

HEINZ 57 SCIENTISTS FOR RESEARCH AND DEVELOPMENT

Heinz need scientific staff for research into food processing and packaging at their new research centre recently opened in pleasant parkland at Hayes, Middlesex. Laboratory and pilot shop facilities are excellent. However, research and investigations cannot be carried out with modern equipment alone. They need more well-qualified scientific staff, specifically:

BIOCHEMISTS · CHEMICAL ENGINEERS
CHEMISTS · FOOD SCIENTISTS
PHYSICISTS · PHYSICAL CHEMISTS
MICROBIOLOGISTS

Work covers most technological aspects of food processing and, in particular, the Physicist/Physical Chemist will be engaged on investigations into technological aspects of food packaging. Applicants should have at least a good honours degree or appropriate Diploma of Technology. Research experience or Ph. D. would be an asset for the Biochemical post. The upper age limit in all categories is 32, although this is by no means inflexible. Salaries and working conditions are those of a large progressive company. Financial assistance in changing location can be granted in certain cases.

Apply in writing, giving a brief personal history, to:
The Personnel Officer,
H. J. HEINZ COMPANY LTD.
Hayes Park, Hayes, Middlesex

ASSISTANT VETERINARY INVESTIGATION OFFICERS

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

At least 6 pensionable posts (in provincial towns mostly in the southern half of the country) for men and women aged 23 or over.

QUALIFICATIONS: M.R.C.V.S.; preference given to those with postgraduate research experience.

SALARY: £1,281 (at 23) to £1,719 (at 32 or over) to £1,962. Promotion to Grade I (£1,962 to £2,381 in 6-7 years (or possibly earlier)) for able officers, with prospects of further advancement. Opportunities may also arise for short-term service overseas.

WRITE to Civil Service Commission, Savile Row, London, W.1, for application form, quoting 266/65. Closing date November 5, 1965. (18)

ENGINEERS, CHEMISTS AND PHYSICISTS

FOR H.M. FACTORY INSPECTORATE MINISTRY OF LABOUR

4 pensionable posts (2 for engineers, 2 for chemists or physicists) for men or women normally aged at least 35, but well qualified younger candidates considered. Appointment ultimately will be in London, but first year may be in the provinces.

DUTIES include giving expert advice in their respective professional fields on problems arising from the administration by the general staff of the Inspectorate of the Factories Act and the Offices, Shops and Railway Premises Act.

QUALIFICATIONS: current corporate membership of appropriate professional body or, for chemical branch posts, first- or second-class honours degree or Dip.-Tech. in appropriate subjects, or equivalent or higher qualification. At least two years' relevant industrial or supervisory experience required.

SALARY SCALE (national): £1,662 to £2,227 (interim). Starting salary may be above minimum for candidates with qualifications/experience of special value. Promotion prospects.

WRITE Civil Service Commission, Savile Row, London, W.1, for application form, quoting S/6257/65. Closing date November 5, 1965. (15)

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

UPPER ATMOSPHERE SECTION

RESEARCH SCIENTIST

Applications are invited for appointment as Research Scientist with the Organization's Upper Atmosphere Section located at Camden, N.S.W.

The appointee will take part in the Section's current research programme which involves fundamental investigations, both experimental and theoretical, of the earth's outer atmosphere, including the ionosphere and magnetosphere. The experimental investigations involve both radio and optical techniques, using ground based equipment as well as rockets.

Applicants should have a Ph.D. degree, or have had appropriate postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

SALARY: Dependent upon qualifications and experience within the ranges of: £A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

Promotion within C.S.I.R.O. to a higher classification is determined by merit.

The appointment will be initially for a fixed term of two years, at the conclusion of which an extension of tenure or an indefinite appointment may be considered.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of a fixed term appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.

to whom applications (quoting Appointment No: 544/21) should be addressed by November 13, 1965.

International Biological Programme

BOTANISTS
ZOOLOGISTS
MICROBIOLOGISTS
CHEMISTS

The NATURE CONSERVANCY invites applications for appointment as SCIENTIFIC OFFICER at Merlewood Research Station, Grange-over-Sands, for ECOLOGICAL RESEARCH in their developing programme at Meathop Wood Experimental Site.

Applicants should have a good honours degree and postgraduate research experience. Starting salary on scale £895 to £1,521. Applications, including names and addresses of two referees, should be sent to the Director, Natural Environment Research Council, The Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancashire, not later than October 29, 1965.

SENIOR LABORATORY TECHNICIAN
(Male)

with experience in bacteriology, is required to join a research group concerned with veterinary studies. Preference will be given to holders of an A.I.M.L.T. qualification, or an H.N.C. in Applied Biology.

The post offers interesting work and valuable experience in a well-equipped modern laboratory and would suit a technician whose main interests are in research.

Applicants should apply in writing, quoting U. 162, to the Administration Officer, Wellcome Veterinary Research Station, Frant, Nr. Tunbridge Wells.

UNIVERSITY OF ALBERTA
DEPARTMENT OF CHEMISTRY

Applications are invited for the position of

PROFESSOR in Physical Chemistry

Candidates in the fields of Solid State, Structure of Liquids, Spectroscopy or Thermodynamics are favoured, but all applications will be considered. Starting salary according to qualifications, in the range $15,000 to in excess of $20,000. Facilities for research are excellent and generous support is obtained from Provincial and National sources. The Department has 30 members and is active in all the major divisions of chemistry.

Further particulars may be obtained from

Professor Harry E. Gunning, Head,
Department of Chemistry,
University of Alberta,
Edmonton, Alta., Canada.

WESTERN AUSTRALIA
STATE PUBLIC SERVICE
MINES DEPARTMENT
GEOLOGICAL SURVEY BRANCH
GEOPHYSICIST, GRADE 2
(Permanent)

SALARY RANGE: £A1,572 to £A2,405 (gross) per annum, according to qualifications and experience.

QUALIFICATIONS: University Degree with Physics as a major subject and Geology as a subsidiary subject.

DUTIES: Geophysical investigations in conjunction with underground water search, in particular bore logging and interpretation.

TRANSPORT: Reasonable amount allowed for transport of appointee and dependent family to Western Australia, provided bond entered into to serve the State for a period of 3 years.

CONDITIONS OF SERVICE: Superannuation benefits, 3 months' long service leave after seven years continuous service, cumulative sick leave entitlements and other general conditions applicable to permanent public servants.

Applications, stating age, qualifications and experience should be addressed by November 5, 1965, to:

Public Service Commissioner,
184 St. George's Terrace,
PERTH,
Western Australia.

DEPARTMENT OF EDUCATION AND SCIENCE
COUNSELLOR SCIENTIFIC—MOSCOW

The Department of Education and Science invite applications from suitably qualified scientists with a wide interest in science and its application for a tour of duty in the post of Counsellor Scientific in the British Embassy Moscow. Russian language, or ability to learn it quickly essential, knowledge of French or German an advantage. The successful candidate will: (1) advise the Ambassador and other Embassy officials on scientific and technical matters (2) promote scientific contacts between Britain and the U.S.S.R., including the exchange of scientists and technologists under the Anglo-Soviet Cultural Agreement and (3) report on trends and developments in science and technology. It will be necessary to gain an understanding of the Soviet system of organization in the scientific and technical field and to establish personal contacts with Soviet scientific organizations. A short briefing tour in this country will be given. Salary scale £3,385 to £3,885, plus allowances to cover the increased cost of living in Moscow.

Application form from B. Johnston, Department of Education and Science, Curzon Street, London, W.1. (Hyde Park 7070, Ext. 314). Closing date for receipt of completed application forms November 7, 1965.

SOUTH WEST WALES RIVER AUTHORITY
APPOINTMENT OF SENIOR INSPECTOR

Applications are invited for the above appointment in the Water Quality and Fisheries Department, based at Head Office, Llanelly. Applicants must possess a degree in chemistry and have had wide experience of water pollution control and the treatment of sewage and trade effluents. Corporate Membership of the Institute of Sewage Purification, in addition to the possession of a degree, would be an advantage. Salary in accordance with Grade A, £1,495 to £1,745. Initial point in scale depending upon qualifications and experience.

Application forms and further particulars can be obtained from Dr. Roscoe Howells at the undermentioned address. Closing date for applications October 30, 1965.

E. A. GRIFFITHS.
Clerk of the Authority.

Penyfai House,
Penyfai Lane,
Llanelly, Carms.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited for the Chair of Physiology which will be vacant on October 1, 1966.

Further particulars may be obtained from the Registrar, University College, Cathays Park, Cardiff, to whom twelve copies of applications (including the names and addresses of three referees), should be sent not later than January 4, 1966.

NEW ZEALAND

University of Canterbury
Christchurch

SENIOR LECTURER OR LECTURER IN MECHANICAL ENGINEERING

Applications are invited for the above-mentioned position. Applicants must hold a degree in Mechanical Engineering, preferably at honours level, of a recognized Commonwealth University, together with either experience in industry, or research and teaching experience at university level. The appointee's principal teaching will be in one of the following branches of mechanical engineering: Aeronautical Engineering, Applied Mechanics or Strength of Materials, according to previous experience. The salary attached to the position is £1,300 to £2,500 per annum, subject to a bar at £2,000 per annum, commencing salary being in accordance with qualifications and experience. (The designation "Senior Lecturer" is used to denote members of staff who are above the £2,000 bar). Approved fares to Christchurch will be allowed for an appointee and his family, together with actual removal expenses within specified limits.

Further particulars and information as to method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in New Zealand and London on November 30, 1965.

OIL PALM AGRONOMIST

SARAWAK

Required to open up and establish a new experimental Station, mainly to be concerned with oil palm and other crops new to Sarawak, e.g., cocoa and hemp.

Candidates must hold a degree in Agriculture and have had postgraduate training preferably with previous experience as an agronomist.

Salary scale, £1,680 to £2,905 a year plus substantial terminal gratuity. Passages provided. Education and child allowances. Government quarters. 24-36 month contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/155/08 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

ASSISTANT CONSERVATORS OF FORESTS

TANZANIA (Tanganyika and Zanzibar)

Required for the administration of normal Districts, including reservations, surveys, enumerations, supervising and directing of harvesting and advice to local authorities on forestry matters. Candidates, between 30 to 40 years, must hold a degree in forestry or a degree in natural science plus a diploma in forestry. Inclusive salary scale £1,281 to £2,757 a year plus 25 per cent terminal gratuity. Education allowances. Generous leave. Passages provided. 21 to 27 months contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC.324/173/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

CHEMICAL ANALYST

A vacancy exists at Pye Cambridge Works Limited for an experienced person for work connected with the control of electro plating processes and techniques of manufacture of printed circuits.

Apply Personnel Manager, Haig Road, Cambridge. Telephone: Cambridge 51351.

THE BRITISH DRUG HOUSES LIMITED

have vacancies for:

PHARMACOLOGIST

PATHOLOGIST/ TOXICOLOGIST

in their Biological Research Department at Godalming in continuance of their expansion programme. Applications are invited from Graduates in Pharmacology, Physiology, Medicine, or related disciplines, who wish to participate in a vigorous programme of research directed towards the discovery of drugs for the treatment of a variety of human diseases.

Generous starting salaries related to qualifications and experience will be paid, with an annual review on a merit basis. Excellent working conditions in modern and well-equipped laboratories are provided and the publication of results is encouraged. The Company operates a contributory Pension and Life Assurance scheme.

Applications, giving full details of academic career and previous experience, should be addressed to:

The Manager,
The Biological Research Department,
THE BRITISH DRUG HOUSES LTD.,
GODALMING, SURREY.

UNIVERSITY OF MELBOURNE

LECTURESHIP OR SENIOR LECTURESHIP in the SCHOOL OF BOTANY

Applications are invited for the above-mentioned positions: those in the field of Ecology will be preferred. Suitable applicants with other research interests will also be considered. The successful candidate should take up duties as soon as possible in 1966. Salary: £A3,250 to £A3,800 per annum. Initial salary will be determined according to qualifications and experience.

Further information, including details of F.S.S.U. type superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 15, 1965.

LIGHTING RESEARCH

A Lighting Engineer (Dip.M.I.E.S.) is required for an expanding daylight laboratory. This post provides scope for original work on prediction techniques and in subjective aspects of lighting. The Department also provides a daylight advisory service for architects: this is a fee-paying consultancy service so projects can be studied in depth. Equipment includes an artificial sky for model studies, and digital computer facilities are available.

No previous experience of daylighting is required, a degree would be an advantage but less important than an interest in interior lighting.

Please write for further information, quoting PAD/L, to L. P. Laidlaw, Pilkington Brothers Limited, Prescot Road, St. Helens, Lancashire.

INSTITUTE OF CANCER RESEARCH: Royal Cancer Hospital. Technician required in Chemistry Department, Chester Beatty Research Institute, Fulham Road, S.W.3, for synthesis of compounds of interest in cancer chemotherapy. Applicants' preferred with experience of heterocyclic chemistry but some knowledge of synthetic procedures in other fields of organic chemistry would be appropriate. Starting salary £750 to £850 per annum.—Apply, with names of two referees, to the Secretary, 34 Sumner Place, S.W.7, quoting ref. 301/B/221.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited from male or female honours graduates in any of the biological sciences including medicine for the post of a Research Assistant in Tissue Culture in the Department of Anatomy, University College, Cardiff. The appointment, for a period of three years, offers opportunities to work for a higher degree. The candidate should be interested in animal cell biology. Laboratory experience, preferably in culture techniques, would be an advantage. The work planned would entail the application of microcinematography, autoradiography and electron microscopy to cultured cells, under the guidance of and in collaboration with Dr. F. Jacoby. Salary £950 per annum. The appointment is subject to the usual terms and conditions of employment of the University College of South Wales and Monmouthshire.

Applications, containing the names and addresses of three referees, should be sent to the Registrar, University College, Cathays Park, Cardiff, and should reach him not later than October 25, 1965.

THE MINISTRY OF OVERSEAS DEVELopment require for their Tropical Stored Products Centre based at Slough an officer for abstracting correspondence and scientific literature in particular the preparation of a card index system on all aspects of stored products. Minimum qualifications: G.C.E. in five subjects including two scientific subjects at advanced level or equivalent; at age 22 pass degree or equivalent. Salary in the Assistant Experimental Officer/Experimental Officer grade according to age in scale £549 to £776 (at age 22) to £1,201 and £1,319 to £1,675. —Applications to the Director, Tropical Stored Products Centre, London Road, Slough, Bucks, to be received by October 29, 1965.

APPLICATIONS INVITED FOR RESPONsible post on technical staff in Physical Chemical Research Laboratories. Duties include responsibility for apparatus stores, ordering and servicing apparatus and equipment, maintaining certain services and training one or more juniors. Salary according to experience and ability within the scales for Universities.—Apply in writing to Secretary, ref.: 7528, King's College, Strand, W.C.2.

INORGANIC CHEMIST REQUIRED either recently graduated or with outside experience. Accommodation available.—Write details Levy West Laboratories Ltd., Bush Fair, Harlow, Essex.

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF MECHANICAL ENGINEERING

Honours graduates in Engineering, Physics or allied subjects are invited to apply for two appointments as S.R.C. RESEARCH ASSISTANTS in the above department for the purpose of carrying out investigations under the direction of Dr. H. E. Rose, Professor of Powder Technology in the following fields of study (1) Electrostatic Precipitation, (2) Dispersion of Solid Particles in Fluids. Duties to commence as soon as possible.

Salary, depending on experience and qualifications, will be up to £1,000 per annum, and the appointments will initially be made for a period of two years.

Application forms may be obtained from the Registrar, King's College, Strand, W.C.2; completed forms must reach him by November 1, 1965.

FOURAH BAY COLLEGE

THE UNIVERSITY COLLEGE OF SIERRA LEONE

Applications are invited for the Chair of Physics. Secondment for a minimum period of two years considered. Salary Scale: £3,000 by £100 to £3,400 per annum. F.S.S.U. Children's, car and outfit allowances. Family passages. Biennial overseas leave. Part-furnished accommodation. Detailed applications (eight copies), naming three referees, by November 15, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom full particulars may be obtained.

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

Applications are invited for two posts in the Department of Biology, at the grade of Lecturer or Assistant Lecturer, one in biochemical genetics and molecular biology, the other in animal physiology. The first appointment will be from as early a date as possible, preferably by April 1, 1966, and the second from October 1, 1966.

Six copies of applications, naming three referees, should be sent by November 15 to the Registrar, University of York, Heslington, York, from whom further details may be obtained.

UNIVERSITY OF LONDON

READERSHIP IN IMMUNOLOGY AT THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

The Senate invite applications for the Readership in Immunology tenable at the London School of Hygiene and Tropical Medicine (salary in the range £2,975 to £3,645 a year).

Applications (ten copies), must be received not later than November 10, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF LIVERPOOL

BUILDING CLIMATOLOGY RESEARCH UNIT

DEPARTMENT OF BUILDING SCIENCE

Wind structure in built-up areas: A recently graduated physicist or electrical engineer is required to take charge of instrument design and application in a study of the turbulence characteristics of the natural wind. The appointment is for two years in the first instance and the salary is negotiable.

For further details, write, giving a brief personal history to the Registrar, The University, Liverpool, 3. Please quote Ref. CV/307/N.

BREWING RESEARCH

Laboratory Assistant, preferably aged between 20 and 23 years, is required for biochemical work in the Research Laboratory of a large brewery group. Please reply with full details of age, qualifications and experience to Chemist-in-Charge, Research Laboratory, Whitbread and Co., Chiswell Street, London, E.C.1.

LIVERPOOL EDUCATION COMMITTEE

LIVERPOOL REGIONAL COLLEGE OF TECHNOLOGY

BYROM STREET, LIVERPOOL, 3

Principal: S. A. J. Parsons, B.Sc.(Econ.), M.I.Mech.E., M.I.Prod.E., M.B.I.M.

Required as soon as possible:

DEPARTMENT OF PHARMACY

POST F/60S. SENIOR LECTURER IN PHARMACOLOGY

Candidates should be graduates (preferably with honours), in Pharmacology, Physiology, Pharmacy or related subject, with a research qualification. The successful candidate will be expected to initiate and direct research to Ph.D. level, for which ample time, laboratory facilities and technical assistance will be available. The successful candidate will be expected to teach the subject to honours graduate level. Salary: £1,895 to £2,115 per annum (under review).

Application forms and further particulars can be obtained from the Director of Education, 14 Sir Thomas Street, Liverpool, 1, on receipt of an addressed foolscap envelope. Return completed forms to the Principal of the College not later than November 1, 1965.

THOMAS ALKER,
Town Clerk.

ASLIB

DEPUTY-HEAD OF RESEARCH DEPARTMENT

The Department is being substantially enlarged, and applications are invited for the new post of Deputy-Head. The successful candidate would have prospects of promotion on the retirement of the present Head of the Department in 1967.

The functions of the department are research, development, consulting and advisory work relevant to the collection, storage and dissemination of specialized information, largely but not exclusively scientific and technical, including investigations on the operations and techniques of special libraries and information services, and the introduction of mechanized systems. The department receives Government financial support. The Deputy-Head would have particular responsibility for planning, initiating and developing research programmes.

Suitable applicants are likely either to have held senior posts in library/information services and to have shown particular interest in library research; or to have had wide experience in the application of research techniques, particularly operational research, in other fields, and an interest in documentation.

The salary scale applicable to the post is: Deputy-Head, £2,335 to £3,192 per annum (Head: £3,385 to £3,885 per annum).

Intending applicants, before making formal application, should write for further particulars to the Director, Aslib, 3 Belgrave Square, London, S.W.1. Final applications should be received by November 15, 1965.

WOOLWICH POLYTECHNIC

LONDON, S.E.18

DEPARTMENT OF MATHEMATICS

Applications are invited for the following: Lecturer or Assistant Lecturer, Grade B. Applicants should hold an honours degree in mathematics and have appropriate teaching and/or industrial experience. The Department is engaged in teaching and research for first and higher degrees of the University of London, for sandwich degrees awarded by the Council for National Academic Awards and for other postgraduate courses. Research by staff is encouraged and the department is well equipped with digital and analogue computing facilities. Salary Scales: Lecturer, £1,670 by £45 to £1,895 per annum; Assistant Lecturer Grade B, £830 to £1,760 per annum, plus London allowance of £45 to £60 (all subject to review).

Application forms and further particulars from the Clerk to the Governing Body, to whom completed forms should be returned by October 22, 1965.

COUNTY BOROUGH OF LUTON

LUTON COLLEGE OF TECHNOLOGY

DEPARTMENT OF SCIENCE

Principal: W. F. Stephenson, B.Sc., F.R.I.C.

Vacancy for January, 1966 for Lecturer in Zoology to teach the subject to the level of B.Sc. (General Honours) and be able to offer a speciality, e.g., Parasitology or Genetics. A good honours degree and preferably some research and teaching experience required. Research activity is encouraged. Salary scale, £1,875 to £2,140.

Send s.a.e. (foolscap) for application form to the Director of Education, 63-69 Guildford Street, Luton.

RADIOLOGICAL PROTECTION SERVICE

(Ministry of Health and Medical Research Council)
Clifton Avenue,
Belmont, Sutton,
Surrey

requires a

PHYSICIST

to assist in the development of techniques for measuring low levels of radioactivity. Knowledge of electronic circuits would be an advantage. Good honours degree required. Salary range £1,125 to £1,700 per annum, plus London Weighting. National Health Superannuation Scheme.

Applications, with the names and addresses of two professional referees, to the Director at the above address.

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSICS

Applications are invited for a post of Technician in a research laboratory. Enthusiasm and wide interests will be required for work including vacuum, low temperature and super conductivity technology. Salary within the range £600 to £840 plus qualification allowances. Minimum age 20.

Applications, in writing, quoting two referees, to Dr. A. F. G. Wyatt, Department of Physics, University of Nottingham.

MIDDLESEX HOSPITAL MEDICAL SCHOOL

INSTITUTE OF CLINICAL RESEARCH

Medical Laboratory Technician required.

Further details from: The Secretary, Institute of Clinical Research, 40 Hanson Street, London, W.1. MUSeum 2241.

UNIVERSITY OF GUELPH

Research Assistantships in Organic, Biological and Physical Chemistry are available for university or R.I.C. graduates to study for the M.Sc. or Ph.D. degree commencing in January, June and September, 1966. Value $2,800 to $3,200.

For details, apply: The Head, Department of Chemistry, University of Guelph, Guelph, Ontario, Canada.

PART-TIME VACANCY FOR A SCIENTIFICally qualified bacteriological assistant in the Enteric Reference Laboratory. Degree should be preferably in a biological subject. The work is mainly concerned with bacteriophage typing. Good opportunities for research.—Applications, giving details of age, qualifications and experience, etc., to: The Director, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

CHEMICAL ENGINEER REQUIRED FOR job with considerable potential. Small Public Company in London area. Salary according to experience.—Box No. 866, T. G. Scott and Son, Ltd., 1 Clement's Inn, London, W.C.2.

MINERALOGIST

A vacancy for a suitably qualified person who would like to make Mineralogy his career exists in the Anglo American Research Laboratory.

The mineralogical section offers facilities for X-ray diffractometric and X-ray spectrographic work and is also extensively equipped with standard optical and photomicrographical apparatus. The work includes the examination of concentrates, minerals, rocks, ores and other materials and provides a unique opportunity for a person interested in the practical application of mineralogy to acquire experience in this field.

Although not a pre-requisite, a Master's degree is a recommendation for the post.

Interested persons are invited to apply in writing to:

The Personnel Officer (Head Office),
Anglo American Corporation of South Africa Limited,
P.O. Box 4587,
JOHANNESBURG.

No Age, Sex or Fitness Qualification

SCRIPT WRITING AND VISUALISATION ON MEDICAL AND BIOLOGICAL SUBJECTS

Permanent appointment exists for biologically/medically knowledgeable person who possesses imagination and skill at communication in both words and visuals, to undertake research, writing and visualisation for film and film-strip documentaries for professional audiences.

Adequate facilities, enthusiastic management support, pleasant London suburban office, negotiable hours of work, flexible ideas on salary.

Attractive remuneration prospects for person who can conquer the creative challenge and deliver documentaries which are intellectually stimulating, accurate, visually attractive, and commercially justifiable.

An appropriately qualified man or woman who possesses some physical disability which precludes most types of employment would be welcome.

Please write for a Personal History Form to the Staff Manager, The Bayer Products Company, Winthrop House, Surbiton, Surrey.

KAGERA NATIONAL PARK
RWANDA

Two experts are required to advise the Rwanda Government on the development of the Kagera Game Park and the adjacent Mutara hunting preserve. One should have wide experience of game ecology and the other practical experience of National Parks Administration. East African experience and a knowledge of Swahili or French essential. Salary £60 to £75 per week according to experience. Candidates should normally be nationals of the United Kingdom or Republic of Ireland. The assignment is for 3 months.

Please apply for further details and application form giving full name, qualifications and experience and quoting RC 275/149/01 to the Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London S.W.1.

SHREWSBURY SCHOOL

Wanted, in September, 1966, a master to teach Chemistry to University Scholarship level.

TECHNICIAN OR SENIOR TECHNICIAN required for clinical research in the Metabolic Unit of the Department of Psychiatry. Appointment at higher grade will depend on previous biochemical experience and qualifications. Whitley Council salary and conditions.—Application forms from the Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5 (Ref. Psy).

MANCHESTER REGIONAL HOSPITAL Board have a vacancy for a male or female Scientific Officer in the laboratories of the Blood Transfusion Service. An honours degree in science, pharmacy or biology is essential. Salary £935 (first- or second-class honours), rising to £1,610, though candidates with postgraduate qualifications or experience may be given a higher starting salary.—Applications should be sent to the Director, Regional Blood Transfusion Centre, Roby Street, Manchester, from whom further particulars may be obtained.

LECTURES AND COURSES

THE AUTUMN MEETING OF THE British Society for Immunology will be held at the Wellcome Building, Euston Road, London, N.W.1, on Friday, October 29 from 10 a.m. until 6 p.m. and on Saturday morning from 9.30 a.m. until 1 p.m. The Payling Wright Memorial Lecture entitled "The present status of research on transplantation" will be given by Sir Peter B. Medawar, F.R.S., at 2 p.m. on Friday. The remainder of the programme consists of open papers.

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF LONDON KING'S COLLEGE
RESEARCH STUDENTSHIP IN METALLURGY

MASS-TRANSFER IN SODIUM SYSTEMS

Applications are invited for a Research Studentship in Metallurgy. The work will be concerned with the mass-transfer of alloying elements, especially carbon, between plain carbon and alloy steels, immersed in high purity molten sodium at temperatures up to 850° C. Experiments will be made both under isothermal conditions, and with a temperature gradient existing in the sodium.

The value of the Studentship will be equivalent to the current S.R.C. rates, i.e., £500 per annum. Applications should be sent to the Registrar, King's College, Strand, London, W.C.2, not later than November 1, 1965, giving age, qualifications, experience and the names of two referees.

ROYAL HOLLOWAY COLLEGE
UNIVERSITY OF LONDON
DEPARTMENT OF PHYSICS

Applications are invited from graduates with B.Sc. or equivalent in physics or mathematics, for a Research Fellowship for theoretical work on non-linear optics in connection with a laser group at the College. Salary scale, £1,300 by £75 to £1,450. Details may be obtained from the Secretary, Royal Holloway College, Englefield Green, Surrey.

Four copies of applications, together with the names and addresses of two referees, should be sent to him as soon as possible.

UNIVERSITY OF SHEFFIELD
DEPARTMENT OF CHEMISTRY

Applications are invited for a Postdoctoral Fellow, preferably with some experience in X-ray crystallography, to collaborate with Dr. B. Stevens in a study of the photochemistry and radation chemistry of crystalline aromatic compounds. Starting salary £1,100 per annum with F.S.S.U. provision. The fellowship will be available for one year in the first instance.

Applications, with the names and addresses of two referees, should be sent to the Registrar as soon as possible.

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BOTANY

Applications are invited for the post of Research Fellow under a three-year grant from the Science Research Council. In association with Dr. A. Myers the study will be concerned with the effect of light on the nucleases of seedlings. Candidates, who should preferably have a higher degree, should have research experience in enzymology. The salary will be £1,050 by £75 to £1,200 plus F.S.S.U. benefits.

Applications, together with the names of two referees, should be sent to the Deputy Secretary, the University, Southampton. Closing date for applications is October 31 and the appointment will be from January 1, 1966.

THE PROPOSED UNIVERSITY OF SURREY
(Battersea College of Technology)
Research Studentships in Metal Physics

There are vacancies for Research Students in the Metal Physics Group in the following subjects:

1. An investigation of the twinning modes in crystalline mercury.
2. Measurement of the thermal conductivity of metals at liquid helium temperatures.
3. The relation between domain wall energy changes and the characteristics of heterogeneous non-magnetic precipitates in a magnetic matrix.

The last investigation will be carried out mainly in the Mullard Research Laboratories at Redhill under the supervision of Dr. K. Hoselitz, Visiting Reader in Physics, in close collaboration with the Department.

Prospective students should possess a good honours degree in physics of a British University. They are asked to write to Dr. K. E. Puttick, at the College, London, S.W.11, giving their qualifications and, if possible, the names of two referees.

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING
RESEARCH FELLOWSHIP IN RADAR AND SIGNAL PROCESSING

Applications are invited for a Research Fellowship to work on new radar techniques involving cylindrical aerial arrays with 360° electronic beam-scanning. Applicants should possess an honours degree in a relevant subject and have completed a minimum of two years postgraduate experience or training. Experience in high-frequency, or radar techniques would be especially useful. Salary will be in the range: £1,050 to £1,200 per annum (exceptionally higher up to £1,400 per annum) with F.S.S.U. benefits. Candidates who do not possess the necessary postgraduate experience may also be considered for a post at a lower salary.

Applications (three copies) naming three referees, should be sent by November 1, 1965, to the Assistant Registrar (Science and Engineering), the University of Birmingham, Birmingham 15, from whom further particulars may be obtained. In reply please quote EE/N.

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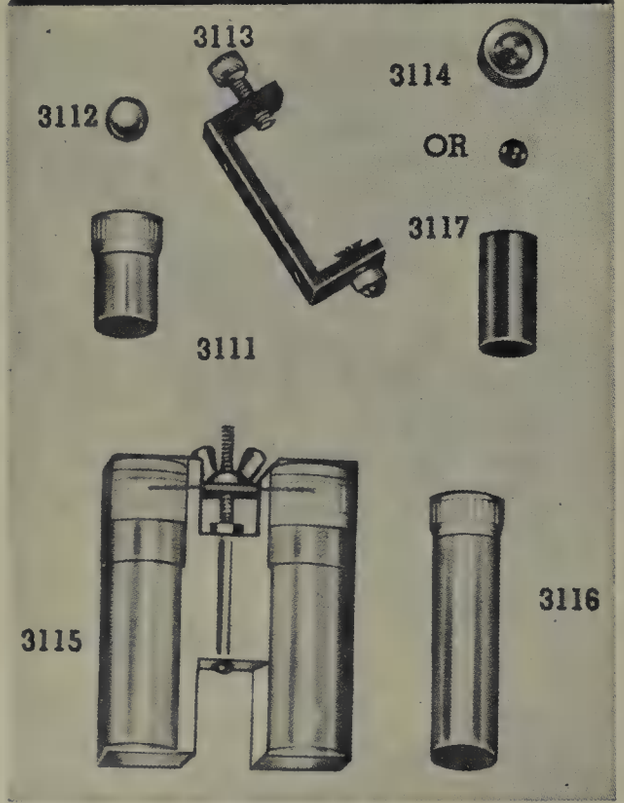

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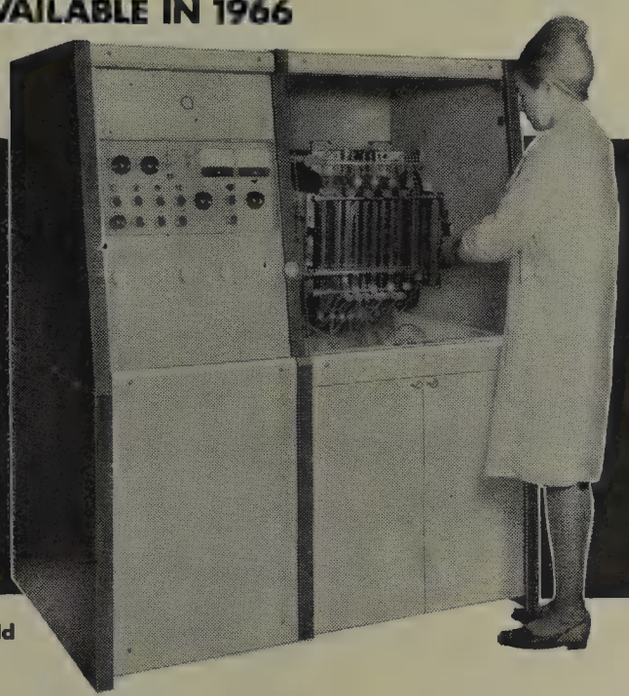

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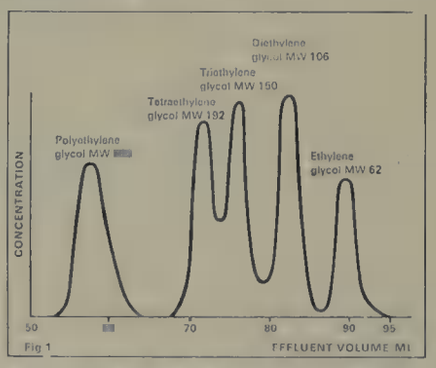
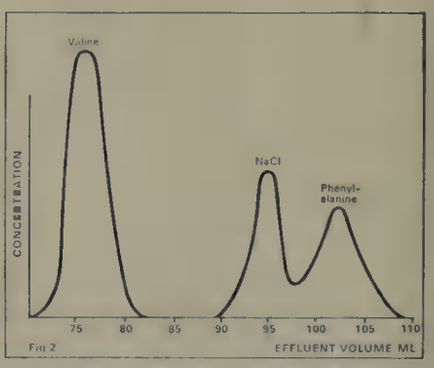


Fractionation of oligomers of ethylene glycol on Sephadex G-10.



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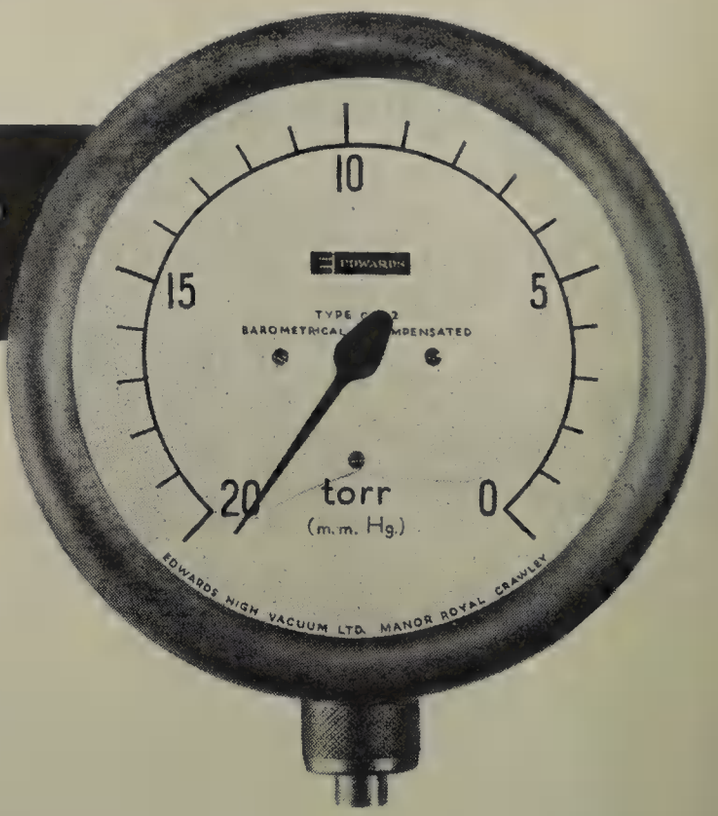

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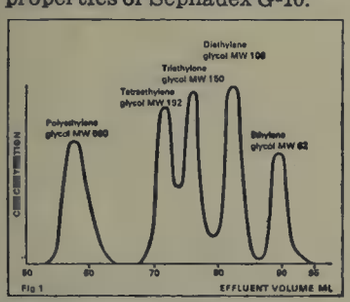

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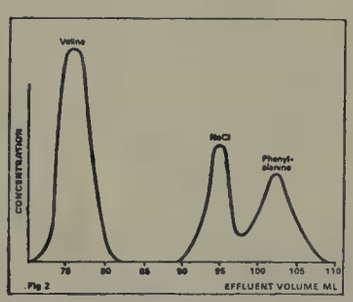

Fig 2 Separation of valine, NaCl and phenylalanine on Sephadex G-10.

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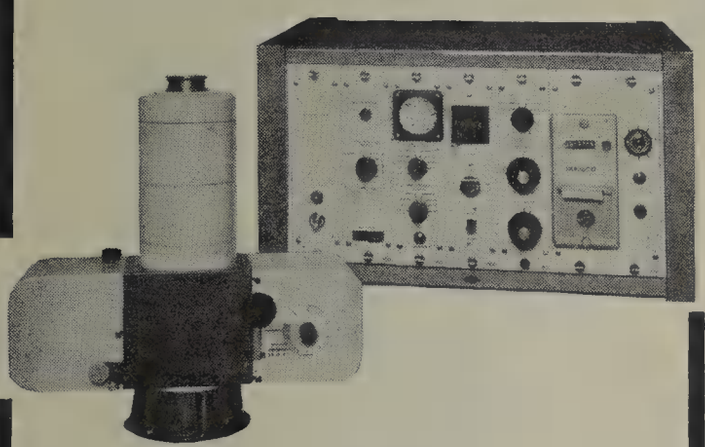

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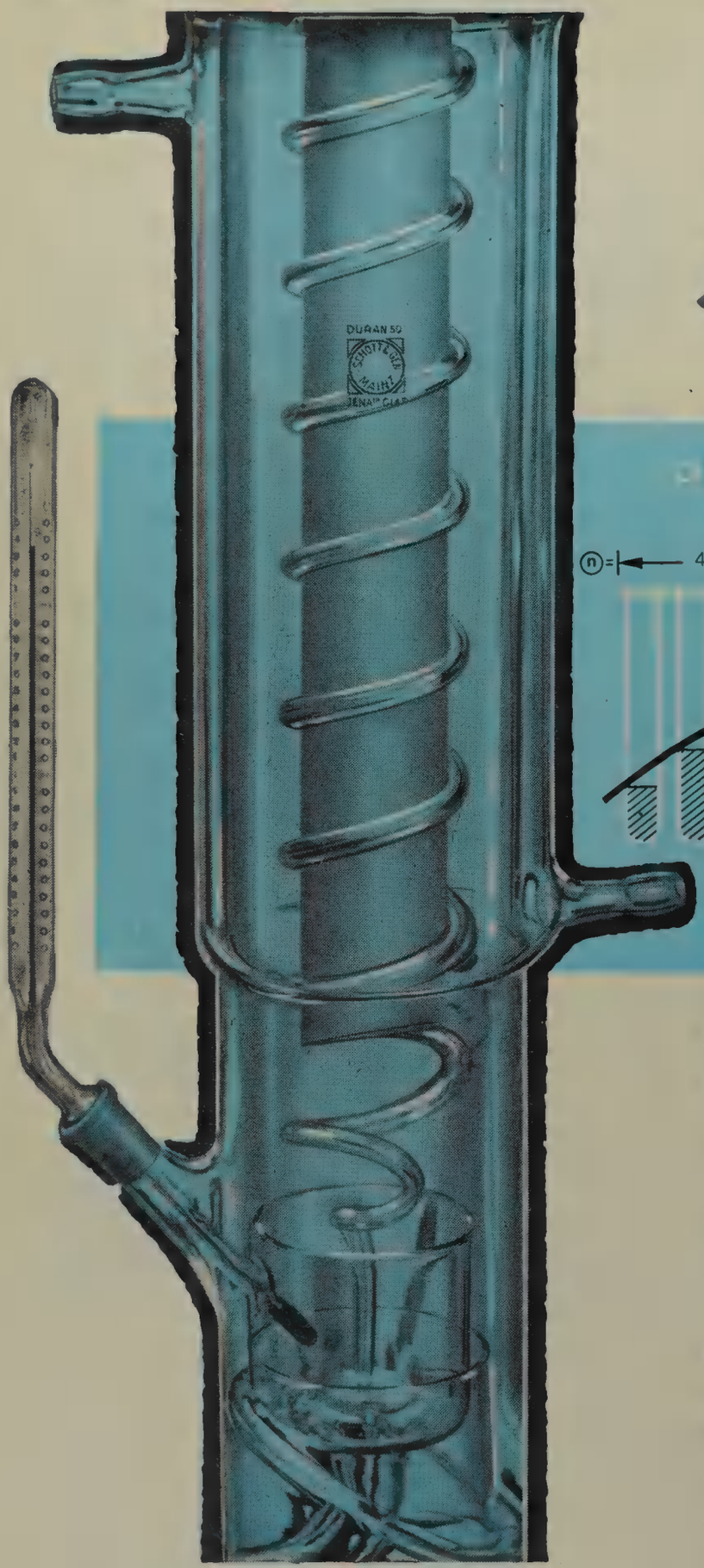

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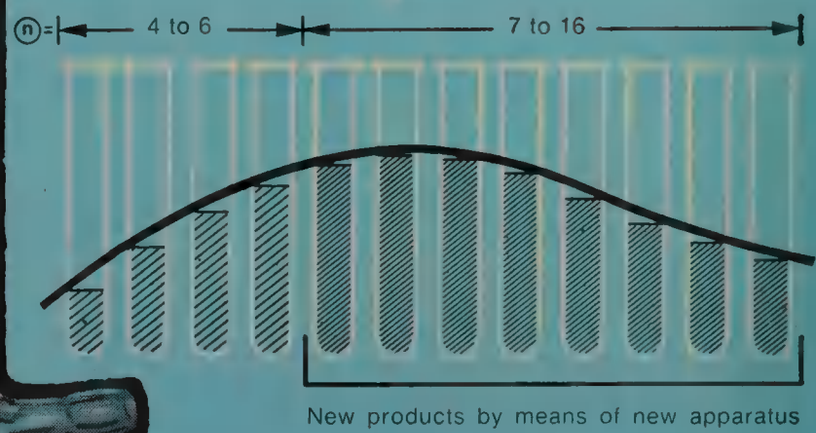

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Service 3: Hilux Base, projection eyepiece

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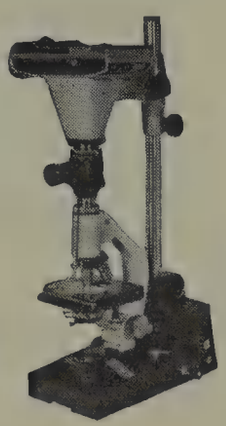
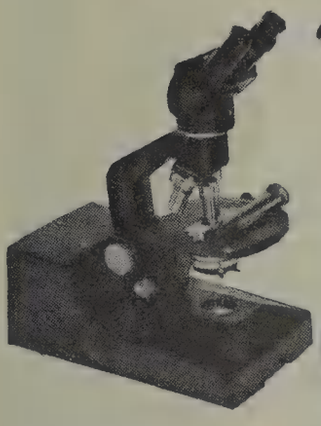


Service 3 with binocular head, Hilux Base



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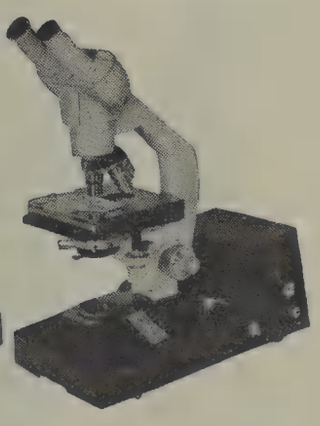

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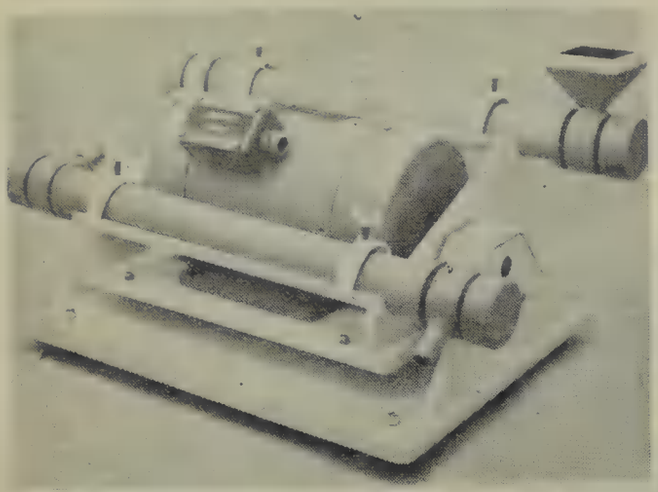

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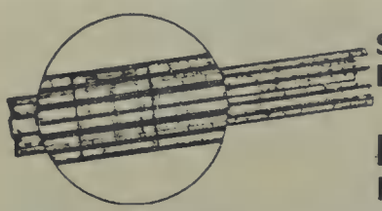

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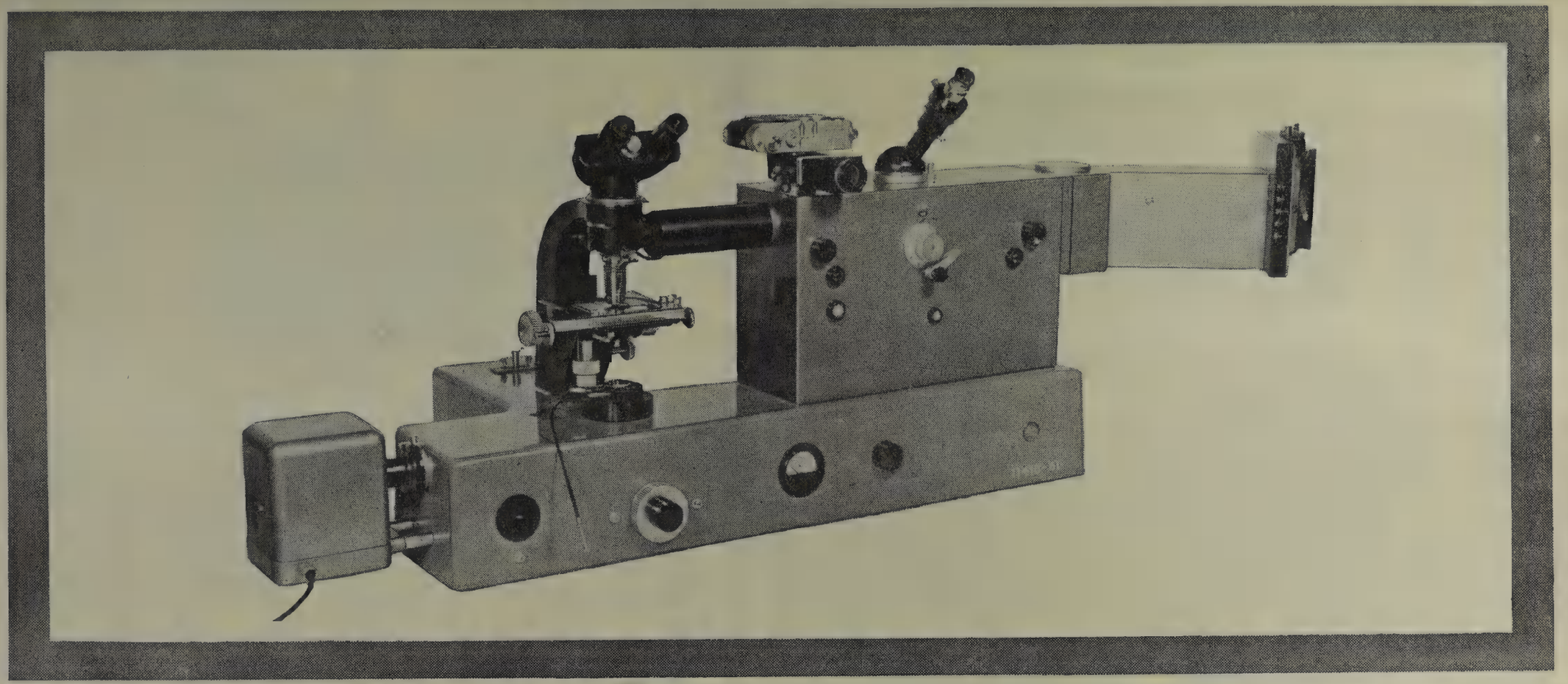

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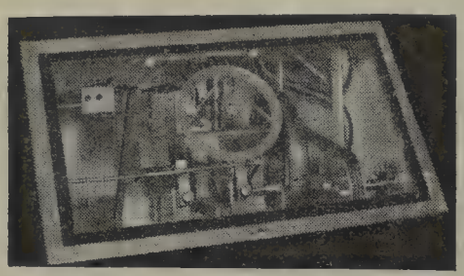

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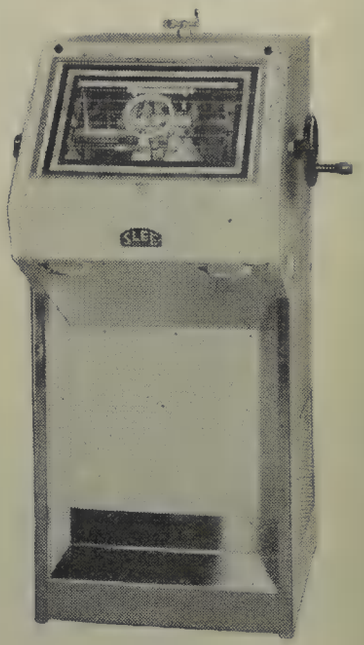

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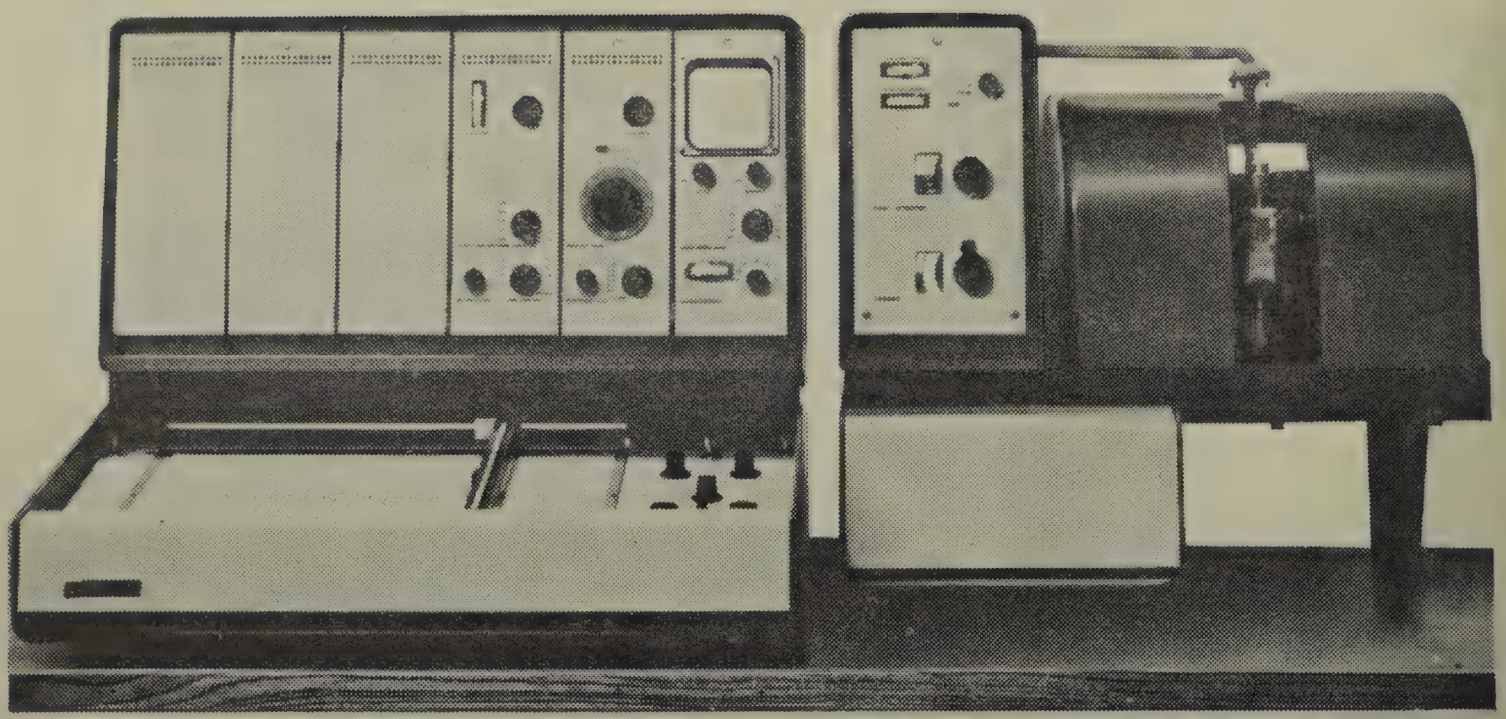

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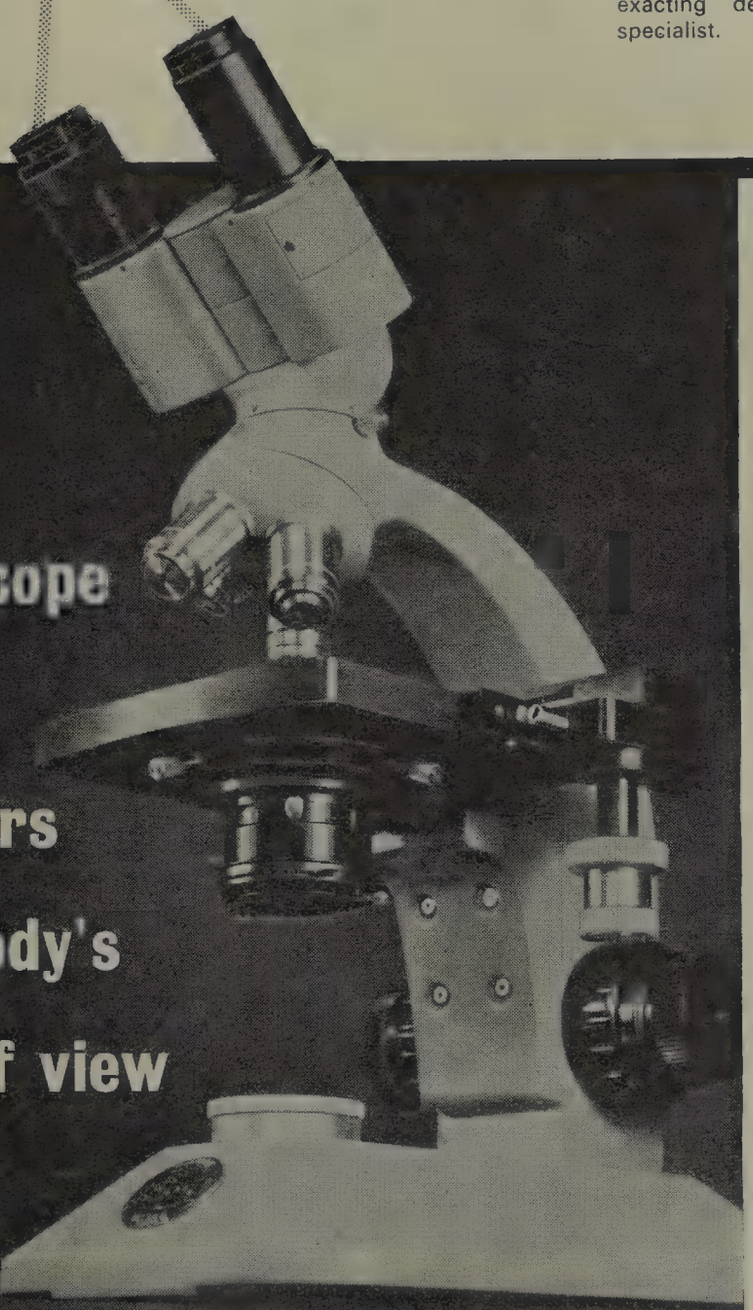

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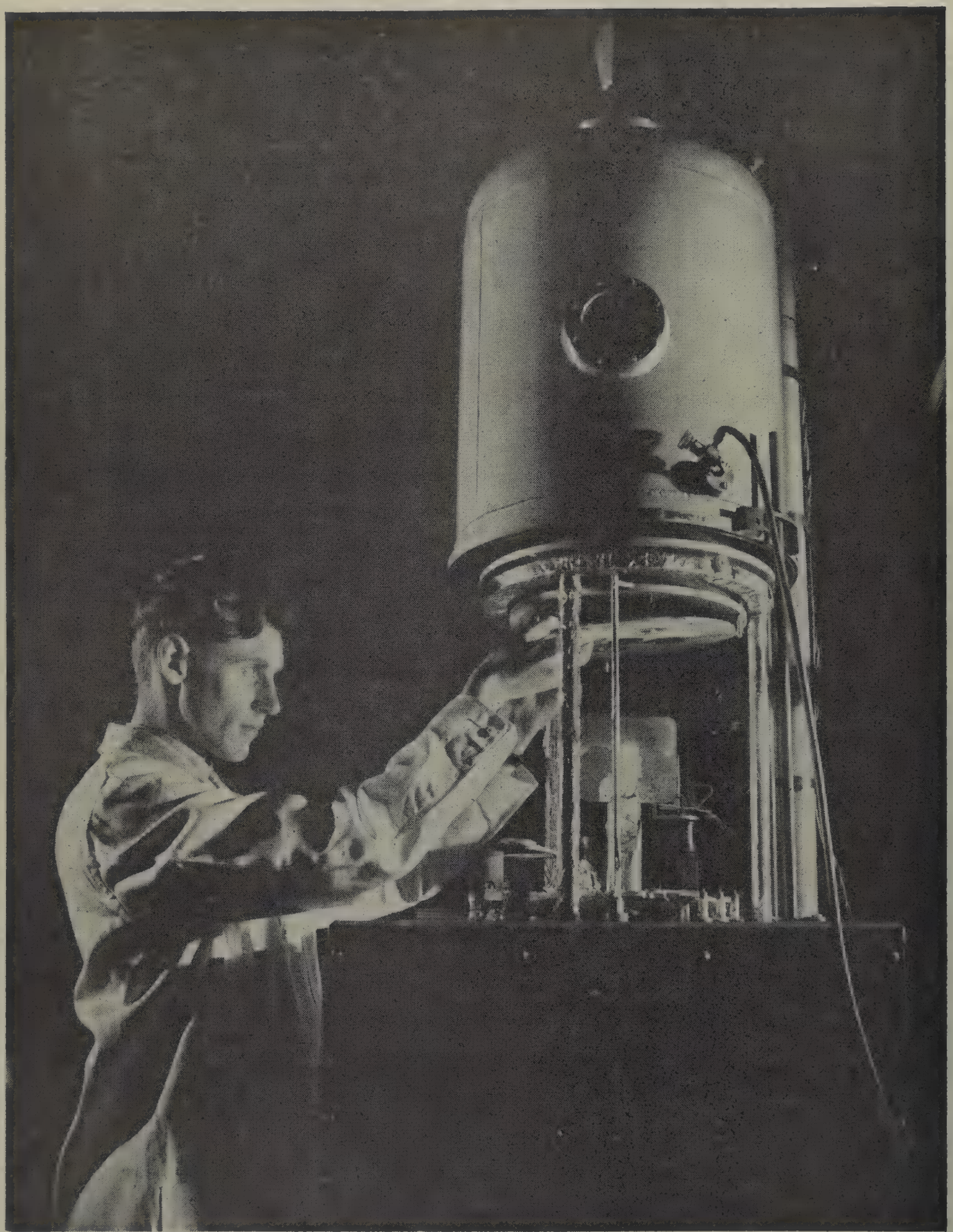

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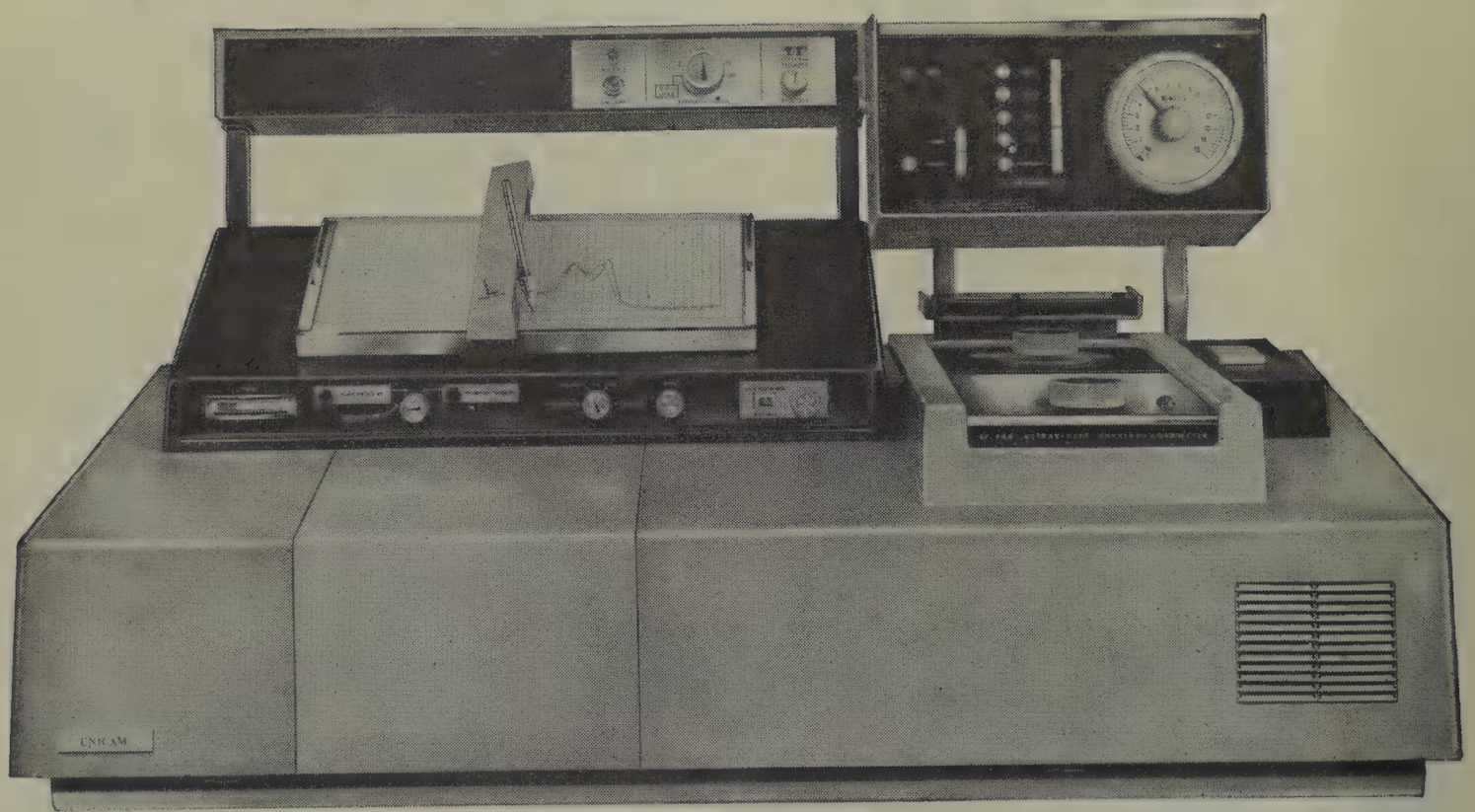

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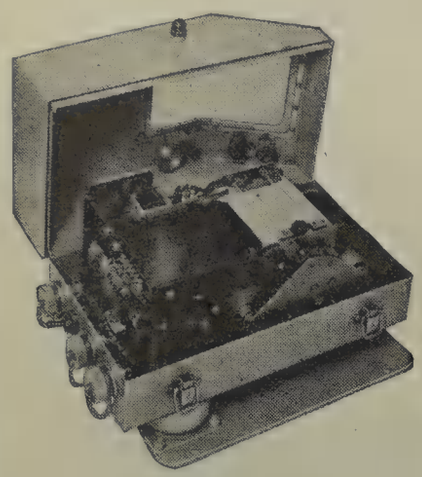

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ALTHOUGH the Civil Service Commission and recruitment to the Civil Service have been mentioned in recent books and pamphlets dealing with Civil Service reform, such reference has been mainly incidental and it has been left for the Estimates Committee in its sixth report for the session 1964–65* to examine recruitment to the Civil Service in any thorough fashion. Even so, the Estimates Committee was indebted to the Acton Society Trust for financing a survey of the attitudes towards an administrative career in the Civil Service of students at a civic university. As the Estimates Committee observes, this underlines the recommendation in its fifth report for this Session that it should be authorized to appoint temporary outside advisers and to obtain assistance from outside—any expenditure involved being borne on the Vote of the House of Commons. Much of the value of the report, in fact, derives not from the Committee's examination of witnesses but from the memoranda which were submitted in evidence by the Acton Society Trust and others—without whose co-operation the scope of the enquiry would have been severely restricted and its authority much diminished.

The enquiry was concerned both with the work of the Civil Service Commission and with the role of the Treasury in recruiting and with direct recruitment by other Departments. The Commission's responsibilities are now limited to the selection and certification of people for permanent posts, under regulations made by the Commissioners and approved by the Treasury. Unestablished posts are now filled by arrangements made by Departments, sometimes assisted by the Commission, and subject also to the approval of the Treasury. The Estimates Committee's recommendations are few, but the implications of the report are far-reaching: the difficulty which the Civil Service has consistently experienced since the Second World War, especially in graduate and professional recruitment, and in obtaining as many recruits as it needs, is set in the perspective of to-day's needs generally. The right questions are asked not simply about recruitment itself but about the opportunities open to recruits and the use that is made of them.

The report deals first with the pattern of recruitment, and here the figures quoted show that since 1954, while needing more recruits, the administrative class only maintained the number of assistant principals recruited and failed to maintain its share of a rapidly increasing field of possible candidates. Moreover, at a time when the fields of university recruitment were widening, the proportion of administrative class recruits from these wider fields was falling, and although until 1963 little difficulty was experienced in recruitment to the executive class, vacancies in some departmental executive classes were not being filled. In the 1964 report of the Commission, however, reference is also made to a shortage in the general executive class—the class which is broadly responsible for the wide range of managerial and administrative functions in the Civil Service and which has traditionally recruited from school-leavers with at least two passes in the Advanced Level Examinations for the General Certificate of Education.

In many respects, recruitment to the specialized classes of the Civil Service, which include the Scientific Civil Service and a range of technical and professional classes, is a lighter problem than that of recruitment to the administrative or executive classes—recruiting procedures being faster and more flexible and often undertaken by the departments. Even so, the Civil Service is at a disadvantage in the competition with other employers, even when the lengthy process of open competition, interview and certification is not needed for temporary posts. Direct appointment by establishments is not possible and Civil Service pay structures do not permit any recognition of the really first-class man on entry. Finally, scientific and technical work in the Civil Service is necessarily more organized than in the universities, which since the Second World War have been one of the main competitors for the best graduates. Certainly, there is not the same freedom of publication, though the rules concerning publication have recently been liberalized.

Of interest in this connexion are some observations on factors influencing the choice of employment in a memorandum from the Civil Service Commission. It is admitted that salaries are much more favourable to the universities than to the Civil Service compared with before the Second World War, but it is considered that attraction to the administrative class lies other than in the cash rewards. In the scientific officer class, the Civil Service has much to offer young graduates in the way of interesting work in their own field with unusually good experimental facilities and reasonable stability in the programme of work, and in general the Scientific Civil Service probably ranks second to a university appointment in the minds of good young scientists. There is, however, a tendency for the young scientist to try a period in industry. To meet this, the Service has in recent years resorted to temporary recruitment direct by departments with subsequent confirmation by the Commission when both sides have made up their minds. Nevertheless, the Commission admits that the Service does not attract as many of the really outstanding young scientists as it would like and that it is possible that this is due in part to the failure of the system of pay on entry to allow recognition of the really first-class man.

In the same context may be noticed some remarks in a memorandum from the Acton Society Trust on possible reasons why graduates from universities other than Oxford and Cambridge do not choose careers in the Civil Service. It is unlikely that the differences can be attributed to different sources of social origin in students, although this may partly explain the popularity of school teaching as a career. The nature of the recruitment process may be a hindrance, and an Acton Society Trust report on the arts graduate in industry pointed to a higher wastage among those who had been recruited into general training schemes than among those who had been recruited for specific posts. Moreover, an applicant for a career in the administrative class is really required to take on the job for the whole of his working life. This may well be a deterrent, as is also the picture of the administrative class as 'the talented amateur', overdrawn though this has always been. Nor has this yet been remedied by the reforms which have followed the Plowden Report, and the Civil Service continues to under-play the need for formal training for members of the administrative class.

* Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964–65—Recruitment to the Civil Service. Pp. xxxviii+269. (London: H.M.S.O., 1965.) 26s. net.

It is at this point that the memorandum touches on the Civil Service Commission itself, which, it suggests, contributes to the present unsatisfactory image of the Civil Service through the rather negative way it handles its recruitment programme for the administrative class. Before examining that criticism of the Commission, some further points in the memorandum on the attitude of students may be noted. The exploratory enquiry conducted by the Trust indicated that some 60 per cent of science students at the University of Hull had no particular careers in mind. Most of the students thought that university teaching and school teaching were the careers most likely to offer a high degree of personal freedom in carrying out one's job, private industry and commerce getting a middle rating, followed by the Scientific Civil Service with the administrative class last. After university teaching, the Scientific Civil Service was regarded as the career most likely to offer opportunity of using the knowledge acquired in reading for a degree, and the administrative class again came last—though it was rated better as regards opportunity for easy transfer of jobs in mid-career. Moreover, except for the nationalized industries, where the opportunity was regarded as equal, between 60 and 85 per cent of the Hull students thought they stood less chance than graduates from Oxford and Cambridge, especially for the administrative class, closely followed by university teaching. More than half the students regarded working in London for the duration of their careers as a definite disadvantage; almost a third were indifferent, and only 12 per cent thought it an advantage. By far the most attractive feature of the administrative class to the students seemed to be its security of tenure, but this and other attractive features did not offset the fact that they gave it a low rating on those attributes which they held to be important in comparison with other jobs.

The extent of the knowledge of the students about the Administrative Civil Service was clearly small. The image of the Administrative Civil Servant which is based on that knowledge is a definite obstacle to recruitment and one that must be rectified. For this the present publicity techniques are inadequate and a new and more direct approach seems necessary. However, it should be noted that the Acton Society Trust thought that the facts about Civil Service recruitment were still extremely scarce and that a full-scale investigation into undergraduates' attitudes towards the Civil Service as a career should be undertaken on behalf of the Civil Service Commission as an essential pre-requisite to discussion. Meanwhile, the shortage of talented graduates and the efficiency of the Civil Service compel the Civil Service Commission to continue and increase its efforts to broaden the appeal of the administrative class. Whatever changes may be made inside the Service itself, the Commission must pursue a more aggressive recruitment policy for this class and do so imaginatively. The criticism is levelled also at the publicity of the Commission and at the university appointments boards. The latter are not so efficient as they might be; they are understaffed, and while they are often ill-equipped to give vocational guidance, for most of them, in quantitative terms, the administrative class is unimportant.

This criticism of the Civil Service Commission appears to have been accepted by the Estimates Committee. In a long memorandum on recruitment to the Civil Service, in which he also suggested that difficulties in recruitment and the like were due mainly to lack of experienced research staff and of money and could be remedied by closer collaboration between the universities, the Civil Service and a Social Science Research Council, Prof. W. J. M. Mackenzie expressed the opinion, based on extensive personal experience, that the Civil Service Commission acts impeccably within its terms of reference. Witnesses before the Estimates Committee both from the Commission and from the Treasury conceded that a change in the range of the Commission's functions had occurred in recent years, which was not envisaged in the original Order in Council, nor provided for in the Order in Council of 1956, from which the Commission derives its authority. The Estimates Committee agrees that there are strong arguments for keeping the related functions of recruitment and selection together within one agency, and believes that little would be gained by the Commission's attempting to restrict itself to the functions contained in the original Orders in Council. It did not pursue the wider question whether the final responsibility for recruiting policy and management should remain with the Treasury or whether the functions of the Commission itself should be extended, as Mr. S. Brittan suggested in his recent book*, and merely note with apparent approval Prof. Mackenzie's comment: "The problem is whether the Commission's terms of reference are right".

Prof. Mackenzie wrote his memorandum before the publication in the winter issue of *Minerva* (3, No. 2, 172; 1965) of Prof. R. J. Stone's article "A Model of the Educational System", which illustrates the type of serious research and analysis which he regards as necessary in answering the questions from the Estimates Committee. He suggests, however, that two vital modern functions are now lost to view in the Victorian tradition which still appears to underlie the work of the Commission. The notion that numbers of corrupt persons are trying to insinuate themselves and their friends into the public service is archaic: in the modern world the Commission is the recruitment section of the personnel division of a large organization. If it cannot find its recruits, it must first look in a sophisticated way at the employment market and at what the organization offers to the market. If candidates of the right type cannot be found, it must report back fearlessly and constructively to the higher level. Secondly, the Civil Service is perhaps unique among lay organizations in having no public relations department, and Prof. Mackenzie suggests that the Commission cannot possibly do its job to-day unless it is able to face its competitors squarely. In his view, its function must be that of a recruitment and public relations department for the Treasury Department, which is now responsible for the personnel policy and general efficiency of the Civil Service. He regards the Commission as a well-run body, but considers that a symbolic break with the Victorian tradition might be an advantage. In this view he differs from the Fabian Group, which in 1964 proposed that personnel management should be transferred from the Treasury to a reformed Civil Service Commission strengthened by new appointments and by staff from the Treasury.

In this long memorandum, Prof. Mackenzie makes many pertinent observations and comments, of which only a few can be noted here. He pays tribute to the outstanding success of the British Civil Service in the organization and management of routine clerical work, but he does not see how its strong executive structure can be maintained except by upgrading the status and salaries of the executive class and its related departmental classes. Challenging the idea of the amateur, he points to some five respects in which the task of the administrator calls

* *The Treasury under the Tories, 1951–1964*. By Samuel Brittan. (Pelican Book A 722.) Pp. 375. (Penguin Books, Ltd., London, 1964.) 6*s*.

for different types of special or professional knowledge and skill. After reminding us that the pool of really good general graduates increases much more slowly than the total output of graduates, he points to five weaknesses in the present intake from the universities. The proportion of candidates with a solid first-class academic record appears to be declining; the net is not flung wide enough; while the proportion of social scientists, particularly economists, is rising, there are very few natural scientists and virtually no technologists; entrants come straight from university to service in a Department and lack training and practical experience of the world; there are few chances of varying the pattern of a career between entry and distinguished success, even of unofficial meeting and argument with contemporaries.

Prof. Mackenzie's comments on the scientific and professional classes offer some constructive guidance to the Commission in pursuing a realistic policy which takes due account of the variations between different fields; if these comments are heeded, they could help to correct the attitude which in the mind of the Estimates Committee is responsible, as much as the general image of the Civil Service, for the poor response in the civic universities. It appeared to the Estimates Committee that the Commission's procedures still concentrate on keeping people out rather than on attracting them. The many advantages which the Civil Service has to offer are not being communicated to the relevant audience, and the Commission has not always translated its understanding of the need for effective public relations into the most effective public relations.

So far as the Civil Service Commission is concerned, the Estimates Committee makes four specific recommendations. Alone, or in conjunction with the Treasury, the Central Office of Information or an outside agency, the Commission should examine the effectiveness of its methods of advertising and publicity. With the Treasury, the Commission should undertake a follow-up survey of executive officers, if necessary on a sampling basis, to determine the efficiency of selection methods and to isolate particular reasons for wastage in the early years of service. The Commission should also seek to quantify the categories of graduates from which entrants to the Civil Service are drawn, and the two posts at principal level recently sanctioned by the Treasury to improve contacts with teaching staff at universities should be used to improve contacts with civic universities as well as Oxford and Cambridge.

Apart from criticizing the approach of the Civil Service Commission to the whole problem of recruitment, the Estimates Committee comments at some length on its advertising and publicity, particularly in relation to the impact of recruitment for the Civil Service on the manpower situation generally—an aspect which incidentally is emphasized in the subsequent White Paper outlining the National Plan. The Committee is also critical of the liaison with both the schools and the universities, especially of the reliance on the appointments boards, the efficiency of which has been questioned both by the Acton Society Trust in its memorandum and earlier by the Heyworth Committee of the University Grants Committee which examined the work of the Boards. It is also critical of delays in selection procedure, the responsibility for which rests as much on the Civil Service as on the Commission itself. The staff side considered that the clerical class was at present losing the best potential recruits because of the time taken to recruit. Mr. B. J. Holloway, in a memorandum, pleaded for the exercise of greater imagination and less rigidity by the Commission. He also credited the Scientific Civil Service with being the only section of the Civil Service that had really faced the problems of competitive recruitment, and pointed out that firms like Imperial Chemical Industries, Ltd., and Unilever could reach a decision in an urgent case on a first-class man in ten days from start to finish. Certainly he felt that the Civil Service could not expect to keep its candidates if it kept them waiting to the present extent.

The Estimates Committee's enquiry involves both short-term and long-term considerations, and in the latter especially are involved the whole structure of the Service and the desirability or not of radical changes in that structure. These issues are raised to some extent in Prof. Mackenzie's memorandum and even more so in one from Mr. D. N. Chester, the Warden of Nuffield College, Oxford. However, although these memoranda are interlocked with some of the questions considered here they are more conveniently considered together with related issues later. Meanwhile, it should suffice to add that, in spite of its specific criticism, the Estimates Committee is confident that the Civil Service's fine tradition of ability and integrity is still very much alive to-day. Moreover, while its structure and its public image may well need review in the light of modern needs, the Civil Service would emerge from such enquiry strengthened in public esteem and in its ability to discharge its responsibilities.

HIGHER EDUCATION AND POLITICS

Eighteen Plus

Unity and Diversity in Higher Education. Edited by Marjorie Reeves. Pp. 226. (London: Faber and Faber, Ltd., 1965.) 25*s*. net.

NO short account could do justice to the many thoughts expressed in this series of essays on the problems which confront the world of higher education in the post-Robbins age. In one sector of this world we are witnessing a crisis in the university more acute than that about which Sir Walter Moberly wrote. Time alone will show the extent to which this crisis can be overcome. It was perhaps one of the inevitable consequences of the Robbins philosophy that the dichotomy between institutions of higher education should be sharpened. This is diametrically opposed to what the Robbins Committee set out to achieve, namely, the integration of higher education. The removal of the colleges of advanced technology from the control first of the local authorities and then of the Department of Education and Science has put these last-named organizations on the defensive—a defensive which is now beginning to take the form of attack.

Mr. A. Crosland, the Secretary of State, in a surprisingly candid speech at the celebration of the seventy-fifth anniversary of the foundation of Woolwich Polytechnic, in April this year, has given a clear indication of Government policy, in which the thinking of the Department of Education and Science is no doubt reflected. In this speech, the text of which has been given very wide circulation, a clear distinction is drawn between the public sector of higher education, "under social control and directly responsive to social needs", and the autonomous sector, by implication oblivious to social needs. This is a harsh judgment on what the universities have accomplished in the years since the Second World War. Mr. Crosland indicated also that the Council for National Academic Awards is to be an instrument of policy in building up the public sector of higher education parallel with the development of the autonomous sector. In my view, there can be only one outcome of the attitude of mind displayed in these philosophies. We shall see, with

the passage of time, growing indignation at the iniquities of selection at eighteen plus and a growing clamour for the autonomous sector to be integrated with the public sector and brought under 'social control'. As Dr. Brian Wilson says in his chapter on the needs of students, in *Eighteen Plus: Unity and Diversity in Higher Education*, "the real issue of the times is not whether we shall have more university education but whether we shall maintain university education at all".

The sixteen contributors to this symposium write mostly on topics which have already been extensively discussed. Most of the papers were, in fact, first given at a conference in September 1963, that is, before publication of the Robbins Report. They deal with a variety of topics within the field of higher education and express many different points of view. These are linked and co-ordinated by a series of interesting commentaries by Miss Marjorie Reeves, Fellow of St. Anne's College, Oxford, who also contributes a chapter on "Prestige". This unsolved problem of the relative esteem to be accorded to different institutions and courses probably does more damage to the cause of higher education than any other single factor.

Dr. G. Templeman describes the considerations which have led to increasing concentration on single-subject degree courses and makes a plea for the enlargement of our notion of university duty so as to cover wider educational responsibilities. "Do students need an ivory tower?", asks Miss Reeves in introducing Dr. Wilson's chapter on student needs. He paints a gloomy picture of threats to university values and refers to the temptation to project an image as a substitute for building a reputation; to dilution by new types of discipline; to the increasingly professionalized attitudes of academic staff; to pressure by clientele with its demand for a qualification and a good time; and to public demand, characterized by growth, status inflation and eventual devaluation. Prof. W. R. Niblett discusses further the influence of expansion on traditional values. Prof. H. Butterfield, in a chapter on the "Springs of Intellectual Vitality", describes the infectiously stimulating atmosphere of the Cavendish Laboratory of the 1920's and what flowed from this. Several contributors refer to the present gulf between the generations which is apparently less acute in the sixth form than at the university. In her chapter on sixth-form studies, Dame Joyce Bishop deplores the stultifying influence of pressure from the universities.

Mr. J. Maitland-Edwards describes the role of technical colleges in higher education, and Miss Monica Wingate the place of the training colleges (now renamed colleges of education). Mr. B. Gowenlock deprecates the "academic rat-race" of the university of to-day. Perhaps the most significant comment is by Mr. D. Jenkins, who points out that education, like war, has now become too important to be left to the experts. This is a stimulating and challenging book which merits study by all who are interested in higher education.

James Cook

FRONTIERS OF CERTAINTY

Beyond the Edge of Certainty

Essays in Contemporary Science and Philosophy. Edited by Robert G. Colodny. (University of Pittsburgh Series in the Philosophy of Science, Vol. 2.) Pp. vii+287. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1965.) 70*s*.

BEYOND the Edge of Certainty is a collection of essays typical of much present-day thought in regions common to philosophy and science. The reader, however, will not be wholly at ease until he recollects what in fact happened to pure mathematics in the nineteenth century. During those years the modern concept of proof was born. To some it may have seemed like an almost endless proliferation of detail: nevertheless, such a view would be wrong. What was emerging was a deeper grasp of fundamentals, and a severe tightening of the screw as to what constitutes validity. To-day, we are witnessing something of the same kind in the realm of physics, a ruthless quest for greater rigour. The search is probably more difficult than before, if only because at some stage Nature will present her bundle of brute facts, and as we try to untie it a number of intractable items always fall out. This is where the strain is greatest between those who, like Dirac, take beauty as the criterion (and indeed this is the more inspiring attitude), and those who maintain that agreement with experiment—although subject to change as technique advances—is at any moment of history the final court of appeal.

One thing is fairly clear: the progressive refinement of laboratory processes and instrumentation will not in itself resolve the dilemma, since the burden of proof lies in the nature of physical law, and the way in which we tend to interpret it. Yet the whole movement is historically conditioned: cosmic physics would be nowhere without Newton and Laplace. Equally, the quantum rises majestically from the matrixes of Hamilton, and the frustration of those who clung to the pattern of continuity to explain the behaviour of radiation. As the editor of this book observes, the pressure is now for 'fine grain', and the right to question everything.

The various authors react as follows: "Newton's First Law" (Hanson) is discussed as a means of entry into natural philosophy; it is not axiomatic in the sense of being self-evident. It needs, as it were, the constant vigil of experience to support its status as a principle.

"The Origin and Nature of Newton's Laws of Motion" (Ellis) reveals their probable Cartesian origin. They can scarcely, therefore, be claimed as a product of induction from experiment: indeed, we have some latitude of choice as is shown by the possibility of constructing different principles of natural motion. In "A Response to Ellis's Conception of Newton's First Law" (Hanson) comment is made on the contrast between kinetics and dynamics, a distinction concerned with how bodies actually move compared with how they would move in certain circumstances. "A Philosopher looks at Quantum Mechanics" (Putnam) finds no satisfactory interpretation as we know the subject to-day. It may still be necessary to go on searching for hidden variables. "The Thermodynamics of Purpose" (Hawkins) is a study in natural teleology; this includes an interesting reconsideration of the green planet Earth, envisaged as a unique heat engine. "The Physics of the Large" (Morrison) looks for a compact theory of the universe, and what constitutes the units of terrestrial life. "Problems of Empiricism" (Feyerabend) is by far the longest article in the series, and leads to the conclusion that the tenet is both incomplete and contains undesirable assumptions. The former limitation is removable if the relation between man and the universe is accepted as a cosmological hypothesis. Finally, "The Ethical Dimension of Scientific Research" (Rescher) brings us face to face with those moral problems which the success of the scientific method has forced on us. It amounts to a deliberate assertion that we are responsible for our talents, and for whatever may follow from their cultivation and deployment.

F. I. G. Rawlins

ASPECTS OF LEARNING

How and Why do we Learn?

Edited by W. R. Niblett. Pp. 196. (London: Faber and Faber, Ltd., 1965.) 21*s*. net.

THIS series of essays springs from a series of public lectures given at the University of London Institute of Education. Publication in book form is perhaps a good way of making some of the lectures by this very distinguished, and very mixed, group of contributors available

to a wider public. But the form, which seems to be becoming more popular, is itself a questionable one. Many of the lecturers/authors are clearly handicapped by the limitations of time and space and one cannot help regretting that Prof. Wiseman and Prof. Morris were not able to develop their subjects more fully. Moreover, this particular collection is an extreme example of the mixture of styles and levels from which this form almost inevitably suffers. The lectures/essays fall into three marked classes: Prof. Wiseman on "Learning versus Teaching" and Prof. Morris on "How does a Group Learn to Work Together" review with great learning, but necessarily in very condensed form, the latest research findings in these fields and support their views with copious references; Dr. Wall, Dr. Taylor and Dr. Kay are more concerned to put forward personal interpretations of their subjects "Learning to Think", "Learning to Live with Neighbours", and "Programmed Learning"; Miss Lee's essay on "Perception, Intuition and Insight" falls half-way between the two groups. Finally we have in Part 2 three personal essays by writers who are not professional 'educationists': Stephen Potter, Lord Caradon and Richard Hoggart.

The student who is looking for a handy summary and authoritative list of sources will probably find the first group most useful. The general reader interested in education will get little from these but much from the second group, while the third group are only marginally related to the title of the collection.

There are important points excellently made in many of the essays; for example, Dr. Wall's warning that an injudicious use of visual aids may tend to fixate children at a pre-conceptual stage and make it harder for them to develop verbal symbolization; or Dr. Kay's suggestion that the next and really important development in programmed learning should come with the instruction of groups rather than individuals. If there is a theme which emerges more than others it seems to be a growing recognition of the importance to learning of relationships within the group and a diminished interest in the paradigm case of an individual in a 'learning situation'. In this respect Dr. Taylor's is one of the most interesting and certainly among the best written of the essays.

The three tail-pieces are all worth reading, though whether it was worth reprinting them in this series seems to me more doubtful. Richard Hoggart deals firmly but justly with the claim of the advertisers that they are really exceptionally honest public benefactors. Lord Caradon's analysis of the genuine concept of trusteeship is sad in its over-optimistic predictions about the future of Cyprus. But the most fascinating is Stephen Potter's revelation of how one man has learnt to enjoy so much of life. How many of us, I wonder, realized that before he turned to gamesmanship he had edited the *Nonesuch Chaucer*?

A. D. C. Peterson

COSMIC RAY PHYSICS

Cosmic Ray Physics

By A. E. Sandström. Pp. x+421. (Amsterdam: North-Holland Publishing Co., 1965.) 80*s*.

During the past twenty-five years a world-wide network of stations has been built up to record the intensity variations of the secondary cosmic rays reaching the surface of the Earth. The detectors comprise neutron monitors which respond to the nucleonic component of the secondary radiation and counter telescopes and ionization chambers which mainly record the mu-meson component. Information on the energy and directional dependence of the intensity variations of the primary cosmic rays far out in interplanetary space is obtained by correcting for atmospheric effects and for the deflexion of the charged particles in the geomagnetic field.

Cosmic Ray Physics, by Sandström, provides a comprehensive account of the methods used in these cosmic ray studies and of the results found. Secondary production and cascade processes in the atmosphere are outlined, and the radiation detectors now in use are described. A detailed account is given of the statistics of particle counting with especial emphasis on the errors involved in the harmonic analysis of the daily variation in intensity. Some difficulty exists in locating the direction of anisotropies in the primary radiation due to the large opening-angles of cosmic ray telescopes and to the scattering of secondary particles in the atmosphere. Further, there is the large dispersion in the magnetic deflexion of different energy primary particles in the Earth's field. Sandström describes what is known about these optical aspects of cosmic ray telescopes.

The well-established intensity variations of the primary cosmic rays are the eleven-year cycle, inversely correlated with solar activity; the Forbush decreases in which a short-term fall of intensity by up to 15 per cent takes place and which may last for a week or more, and the diurnal variation of approximately 0·2 per cent in amplitude. There are also variations correlated with the twenty-seven-day period of solar rotation. Sandström describes the variations, in particular giving a detailed account of the different types of onset and decay of Forbush events. Various theories to explain these primary particle modulations are briefly outlined. Sandström favours the solar beam model in which plasma from the Sun streams out, carrying with it a rather regular, frozen-in magnetic field. Owing to the plasma motion a terrestrial observer also sees an electric field. Forbush decreases are interpreted in terms of particle energy-loss in the electric field together with a screening effect of the magnetic field in the beam. Some early space-probe observations are used to support this interpretation. Experimental results on the daily variation indicate a small excess of particles coming from the evening side of the Earth. Various theoretical interpretations are advanced, including the possibility that this simply represents cosmic rays co-rotating with a large-scale magnetic field, attached to the Sun, but extending far out into interplanetary space. The propagation of solar flare particles to the Earth is discussed in terms of the solar beam theory. Rather short chapters are devoted to extensive air showers, the problem of the origin of cosmic rays and the radiation trapped in the magnetosphere.

The aim of much cosmic ray work is to understand the interaction between the energetic particles, fields and plasma in interplanetary space. Direct measurements are now made of all these physical quantities using vehicles such as the *Interplanetary Monitoring Platform* (*IMP*) series of eccentric orbit satellites. An important early result from *IMP* is the confirmation of the idea that the interplanetary field can be roughly represented by a spiral lying in the ecliptic plane. However, the sea-level recording on which Sandström mainly concentrates yields important complementary results to the satellite work, especially with regard to the accurate measurement of small variations occurring over long periods.

The book may be recommended to undergraduates taking a special course in cosmic rays and to graduates and research workers in the field.

J. J. Quenby

SEDIMENTARY STRUCTURES

Atlas and Glossary of Primary Sedimentary Structures

By F. J. Pettijohn and P. E. Potter. Pp. xv+370 (including plates). (Berlin, Göttingen, Heidelberg and New York: Springer-Verlag, 1964.) 59 D.M.

The first concern of all geologists is to describe logically and accurately the effects wrought by past events in the history of the Earth. Any tool that assists the professional geologist or student in training in this task is therefore to be welcomed. With their book *Atlas and*

Glossary of Primary Sedimentary Structures, Prof. F. J. Pettijohn and Prof. P. E. Potter make a notable addition to the resources of undergraduates reading earth science and of teachers in the field of soft-rock geology. The *Atlas* will also be valued by those professionals whose work lies largely outside the stratigraphical and sedimentological fields.

Most of the book is taken up with 198 photographic plates illustrating many different kinds of sedimentary structure, the majority or which are dependent on current action or on deformation of the sediment prior to lithification. Most of the plates represent natural or artificial rock-exposures, but twenty-nine come from the field of present-day sedimentary environments. The plates are preceded by a brief statement in which primary sedimentary structures are defined and classified. After the plates comes a glossary of names and terms commonly applied to sedimentary structures. The text and descriptions of the plates are also given in German, French and Spanish.

The scope of the book is a very fair measure of the advances in descriptive knowledge of sedimentary structures made since Sorby, in Britain, and Hall. in the United States, began work in this field more than a century ago. The authors' aim is descriptive and the adopted classification of structures logical within the self-imposed limitations of a field guide. Notwithstanding, a classification based on genesis of the higher ranks of structure will be regarded by many teachers as equally valid within these same limitations, on the grounds that the description and analysis of a body of rock should be so arranged as to provide the most direct path to an interpretation. Thus, for example, load casts were perhaps better illustrated with ball-and-pillow, ripple marks with cross-stratification, and current crescents with flute marks, than with the structures chosen as immediate neighbours for them in the *Atlas*.

Although the book is wide in scope there are important omissions. Aeolian dunes and related phenomena are treated very sketchily, although a wealth of photographic data exists for this important group of structures in both North America and Europe. A much fuller treatment of trace fossils could have been given without the authors having risked over-specialization. Many teachers will regret that there is no discussion of the limitations of rock-exposures as evidence of the complete form of sedimentary structures. In the case of cross-stratification, for example, surely some of the many plates used to illustrate this structure could have come from outcrops that provided more than one plane of section. Such evidence is all too rare, but because three-dimensional views are provided is to be valued the more highly.

The *Atlas* is well produced and the publishers are to be congratulated. The plates are excellent technically, if over-abundant in the case of certain structures, and the number of printer's errors is relatively small. However, it is a pity that a text is not provided in Russian, in view of the large amount of sedimentological work published by Soviet scientists in recent years. None the less, Profs. Pettijohn and Potter have performed a notable service, and their book is to be warmly recommended to teachers and students alike.

J. R. L. Allen

A JOURNEY THROUGH MODERN BIOCHEMISTRY

Essays in Biochemistry

Vol. 1. Edited by P. N. Campbell and G. D. Greville. Pp. xi+170. (Published for The Biochemical Society by Academic Press, London and New York, 1965.) 18*s*. 6*d*.

THE major purpose of *Essays in Biochemistry* is to provide the advanced student with an annual group of essays, less detailed than the usual specialist reviews, at a price that is within the scope of students' budgets. In addition, the editors hope the essays will be helpful to teachers and research workers as a vehicle to transport them to sectors of the biochemical front where they are not personally engaged. With the ever-increasing diversification and pace of biochemical research, the second objective seems at least as important as the first.

Volume 1 opens with an account by H. G. Wood and M. F. Utter of the role of carbon-dioxide fixation in metabolism. The three main mechanisms of carbon-dioxide fixation, which involve formation of carbon–carbon bonds, are described and the metabolic significance of such reactions in gluconeogenesis, fatty acid synthesis and metabolism of propionate is critically assessed. The second essay, by R. E. Davies, describes the gradual elucidation of the mechanism of muscular contraction. Although the author begins with the ancient Greeks, he rapidly progresses through the important pre-war work of R. Hill, K. Lohmann, W. A. Engelhardt and others to the massive effort which has been undertaken in the past two decades and finally to his own detailed theory. One is left with the impression that the time is ripe for a determined effort to investigate the mechanism of muscular contraction at a molecular level. The important problem of determining nucleotide sequences in nucleic acids has been surveyed by K. Burton. After describing the analytical methods used for the separation and determination of fragments of nucleic acids, the author discusses enzymatic and chemical methods of degrading nucleic acids and determining the sequence of oligonucleotides. The prediction that the complete sequence of transfer ribonucleic acids will soon be known has already been vindicated by Holley *et al.* (*Science*, 147, 1462; 1965). The remaining articles on oxidative phosphorylation (D. E. Griffiths) and the photosynthetic electron transport chain in plants (R. Hill) are closely related. These are difficult subjects, both for the student and the research worker, and the authors have produced excellent accounts of them by leaving out a lot of detail and work which is of nebulous significance at the present state of our knowledge. The final article in particular is written in a refreshing style; it requires considerable courage to summarize a lifetime's work in a few pages and devote a significant proportion of the available space to indicate the problems that remain to be solved. R. Hill's account of the knowledge that accrued from his early work on haematin and haemoglobin should be an object-lesson to young research workers to familiarize themselves with work that is proceeding in fields contiguous to their own.

For me, it has been a rewarding experience to journey through these carefully distilled accounts of present-day important topics to within sight of our present horizons.

D. T. Elmore

ENGLER REVISED

Syllabus der Pflanzenfamilien

Von A. Engler. II Band: Angiospermen–Ubersicht über die Florengebiete der Erde. Zwölfte völlig neugestaltete Auflage. Herausgegeben von Prof. Hans Melchior. Pp. 666. (Berlin-Nikolassee: Gebrüder Borntraeger, 1964.) 110 D.M.

ENGLER'S *Syllabus* has been a valued work of reference for systematic botanists since its first publication in 1892. With the appearance of the present volume covering the angiosperms the twelfth edition is completed, the volume dealing with the wide range of organisms from bacteria to gymnosperms having been published eleven years ago.

The volume has been completely rewritten by eleven contributors, the whole being edited by Prof. Hans Melchior. The numerous illustrations are all new, the old blocks having been destroyed during the Second World War. Many of the well-known, one may almost say

familiar, features of the original work are retained: the information about economic and, particularly, medicinal plants, the extensive use of *Unterreihen*, and so on. There has, however, been a considerable rearrangement of the major taxa, the most conspicuous feature of which is the transfer of the Monocotyledoneae to the end. Perhaps of greater significance is the fact that the Pandanales, with which the original Engler sequence of orders started, are now placed near the end of the monocotyledons, presumably an acceptance of the view that the apparent simplicity of their flowers is the result of reduction, not an indication of primitiveness.

In curious contrast to this is the retention of the apetalous dicotyledons in the traditional order at the beginning of that group, in spite of their syncarpous ovaries and the widely held view that anemophily is a secondary development in flowering plants. There is thus an apparently unexplained inconsistency between the principles adopted for classifying the monocotyledons and the dicotyledons. This is perhaps not very important. What does seem to be of much greater importance arises from the old, but often overlooked, question: "What is the purpose of this classification ?". The original *Syllabus* is the basis for the arrangement of many large herbaria and numerous Floras, and so forms a guide to them; it is also so familiar to most taxonomists that when they want to look something up they rarely need to refer to the index. The new edition has, at present, neither of these merits and, because of the labour involved, no large herbarium will be rearranged according to it, so it will still be necessary for a working taxonomist to be familiar with the old Engler system and the Bentham and Hooker system, and perhaps some others.

The urge to 'improve' systems of classification by making them more 'natural' is one to which many, perhaps most, taxonomists succumb sooner or later. It is an understandable and perhaps laudable urge, but it seems to me unfortunate when it is given rein in a standard work of reference. In such works, for practical purposes an unchanging classification, even though rather artificial, is the most convenient. It is interesting to see that the differences between the Engler and the Bentham and Hooker systems, formerly so great, are now diminished.

In spite of this drawback, the twelfth edition of the *Syllabus* is a most valuable reference work, with its admirably clear descriptions of orders, sub-orders, families, sub-families and tribes, and the brief, but often illuminating, mention of important genera and species. It is particularly useful to have notes on pollen morphology, embryological characters and anatomical peculiarities included in the family descriptions. The bibliography is extensive and up to date, though perhaps rather deficient in its coverage of Russian literature. There are, inevitably, a few misprints, for example, *Astrodiscus* for *Astrodaucus* and *Bifora radicans* for *B. radians*; otherwise the book is well printed and the illustrations are clear and informative. It is certainly a work to keep near at hand.

T. G. Tutin

PERISHABLE RICHES

Fish as Food

Edited by Georg Borgstrom. Vol. 3: Processing, Part 1. Pp. xiv+489. 125*s*. Vol. 4: Processing, Part 2. Pp. xiv+518. 132*s*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.)

In the preface to Volume 4 of this book the editor says that fish as food is part of the epic of mankind. But, he points out in Volume 3 that: "Only through wise utilization and efficient processing is it possible to save these extremely perishable riches of the oceans and fresh waters and place them within reach of the consuming public independent of seasons and of fishing fortunes . . .".

After death, fish deteriorates extremely quickly: even when packed with ice, biochemical changes commence immediately as indicated by the progressive accumulation of dimethylamine in the flesh. For reasons not yet understood calamitous breakdown of fish catches may sometimes occur to produce, for example 'jellied' plaice or 'milky' hake. When environmental temperatures rise above 0° C spoilage can convert a "bin of sound fish to a soup or porridge. . . ." in 24 h.

The value of fish as food for man and its perishable nature have led from ancient times to the use of methods of preservation. Until comparatively recently, salting, drying and smoking were used. In addition to these techniques increasing use has been made since the last century of cooling and canning, and more recently of chemical additives.

No method of fish preservation is free from possible hazards. More than 4,000 years ago a Chinese wrote about the red discoloration of sea-water when it was allowed to stand and evaporate. This discoloration is due to the 'red-halophil' bacteria which can grow in high concentration of salt and are the cause of the repulsive smell which sometimes develops in salted cod. Whereas most foods keep as well at temperatures up to 5° C as at 0° C, this is not the case with fish, the bacterial contaminants of which begin to flourish as soon as temperatures rise above freezing point. Preservation of fish by cooling therefore requires special means of refrigeration.

By and large, man has been successful in preserving fish so as to allow its wide utilization as food. Fish sauces, fish pastes, marinades, anchovies and other products have become established either as essential or luxurious items in the diets of different countries. The biological mechanisms of many traditional processing methods, and even the composition of some fish products, are not known. The techniques have evolved, not by the application of scientific knowledge, but from trial and error or by force of circumstance. The high cost of salt about 200 years ago, for example, probably led the people of northern Sweden and Norway to use less of it than was needed to prevent growth of bacteria. As a result fermentation occurred and, by good chance, gave a product, fermented herring, which is harmless to eat and has become prized as a delicacy.

The practice of food technology is increasing rapidly, and as it does so much new knowledge and many scientific and technical problems become apparent. This is particularly so in the case of fish processing; the discussions contained in the two volumes under review reveal the complexity of the problems and also that much is being done to try to resolve them. It is not enough to know that our forefathers preserved fish by smoking them in the household chimney. On the scale that smoking of fish is now required it is necessary to know about the chemical and physical nature of smoke itself, the costs of producing it, and how temperatures in smoke kilns can be controlled. The fish sauce of Vietnam is known in France as an exotic addition to the diet. But, in a world facing catastrophic shortage of food, the product may have much greater sigificance for, as revealed by modern analytical techniques, it contains appreciable amounts of essential amino-acids. Until about thirty years ago the watery by-product obtained in the process of making fish meal was discarded. But the release of this waste not only caused serious pollution, but was a loss of valuable material. Fish solubles are now used as additions to animal feeds; they promote growth not only because of their vitamin content, but also because of the presence of a 'fish factor' the nature of which is unknown.

These are but one or two examples of the many topics discussed by the different authors of the two volumes, which bring to completion the treatise, *Fish as Food*. Dr. Borgstrom is to be congratulated on editing, and substantially contributing to, a work which will surely remain for many years the standard exposition of the subject.

G. R. Wadsworth

The Basic Laws of Arithmetic

Exposition of the System. By G. Frege. Translated by M. Furth. Pp. lxiii+143. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 40*s*.

IT is scarcely surprising that Frege's two great works, the *Grundlagen der Arithmetik* (1884) and the two-volumed *Grundgesetze der Arithmetik* (1893, 1903), made little contemporary impression. Frege's view, that mathematical propositions are truths of pure logic, ran contrary to accepted philosophical opinions of the day; his style, partly because of the severely abstract topic, was crabbed, and his symbolism idiosyncratic. Russell's almost accidental discovery of Frege's work, and his celebrated antinomy which struck away the basis of much of Frege's elaborate construction, gave due recognition to Frege as a great pioneer in the field of mathematical logic. Mr. Furth provides a new translation of the introductory parts of the *Grundgesetze*, with the appendix in which Frege somewhat pathetically announces the receipt of the Russell paradox just as his own work was completed and sets about trying to repair the damage. An introduction of some 50 pages discusses Frege's work and explains his themes, his language and symbolism, and adds a very necessary account of the way in which certain technical terms have been translated. Frege hoped to start with a small number of logical truths and a small number of rules of inference, and thence derive all the superstructure of arithmetic and analysis. Perhaps to-day there is less interest in building the superstructure than in examining the logical foundations, and Frege's semantics and his whole philosophy of language seem more relevant to modern enquiry than his inferred consequences.

A mathematical classic has been defined as "a book which must be written but need not be read"; it may be asking too much of modern students that they should read the originals, but Mr. Furth gives them a good opportunity of understanding why books such as Frege's had to be written. T. A. A. Broadbent

The Application of Continued Fractions and Their Generalizations to Problems in Approximation Theory

By A. N. Khovanskii. Translated by Peter Wynn. (Library of Applied Analysis and Computational Mathematics.) Pp. xii+212. (Groningen: P. Noordhoff, Ltd., 1963.) n.p.

THIS book is written in a pleasant and easy style. It is a welcome addition to the sparse and inaccessible literature on the application of continued fractions to approximation theory. The advent of digital computers has renewed interest in this subject as in many situations it provides a more efficient form of approximation than power series or other forms. The book provides a suitable introduction to the larger and more theoretical treatise on the subject by H. S. Wall.

The first chapter (75 pages) is introductory; it describes various notations and basic concepts: convergents and the related recurrence relations, equivalence between continued fractions and power series and their transformations, convergence and its domain, and convergence tests for various forms of continued fractions.

The second chapter (75 pages) derives a continued fraction expansion for the solutions of various differential equations of the Riccati type. This leads to the expansion in continued fraction form of a comprehensive list of basic mathematical functions: binomial, trigonometric, exponential, logarithmic, hypergeometric, Bessel's, gamma functions and others.

The last two chapters describe various further techniques such as the derivation of continued fractions expansions using Cesaro summation methods and the use of matrices to describe generalized recurrence relations and their convergence. I. M. Khabaza

Determination of *p*H

Theory and Practice. By Dr. Roger G. Bates. Pp. xv+435. (New York and London: John Wiley and Sons, 1964.) 100*s*.

THIS book is a revised and enlarged version of *Electrometric p*H *Determinations* by the same author. The scope of the book has been extended to include non-instrumental determinations of *p*H and these are fully surveyed and contrasted with instrumental measurements.

The early chapters of the book are devoted to the various definitions of *p*H scales from theoretical and practical points of view; these are extended to part-aqueous and non-aqueous systems. The treatment of the theoretical aspects is clearly stated with concise definitions of terms, symbols and conventions in accordance with the International Union of Pure and Applied Chemistry recommendations. A discussion of the choice and use of secondary standards is included in a chapter on *p*H standards, while the primary standards are fully defined and tabulated. The performance of primary standard solutions is usefully summarized.

Various forms of electrodes used with *p*H instruments are fully discussed. These include hydrogen, quinhydrone, antimony, silver/silver chloride, calomel and glass electrodes. Understandably, the various commercial electrodes and types of *p*H meters reviewed are mainly of American origin.

The book is concluded with a short section on the principles of automatic control using *p*H sensing systems. Regrettably, a number of useful tables are lost in the general text of the book; one would have expected such tables as those denoting *p*H values of standard buffer solutions to have been included in the appendixes. In these days of high cost books this is remarkably good value for money; it is clearly presented and readable.

R. Sawyer

L'Homme Face à Son Destin

Par Charles Léopold Mayer. Pp. 251. (Paris: Éditions Marcel Rivière et Cie., 1964.) n.p.

IN this little volume the reader will find the results of a philosopher's thoughts extending over a quarter of a century. It is distinguished by its lucid French logic, combined with a certain classical inevitability of argument. There are thirty-four short chapters, and their general trend can be seen as an attempt to expose the main facets of human experience, giving due weight to both sides of a question. For example, no absolute truth can be claimed, but only relative truth, and one law to govern the universe (necessity), or failing that, the law of chance. An interesting quasi-mechanical principle is introduced, namely, that of "least irritability" which controls the behaviour of the living world much as Maupertuis's law of least action functions in dynamics. For the author, Nature is amoral, but axiological judgments belong to human beings. It may well be, too, that natural law is a useful and necessary fiction, though no less a person than Leonardo da Vinci venerated it, and in terms of it made both artistic and technical progress.

If an estimate is to be made of the value of a collection of meditations such as this, it is perhaps prudent to recommend it as an academic study, since its conclusions are significant, but not immediately applicable to the daily round and common task. Much of it is in the style of soliloquy, very attractively expressed in the French idiom.

F. I. G. Rawlins

The Thymus in Immunobiology

Structure, Function, and Role in Disease. Edited by Robert A. Good and Ann E. Gabrielsen. Pp. xxii+778. (New York and London: Hoeber Medical Division, Harper and Row, 1964.) 24.50 dollars.

THIS volume is based on the proceedings of the first International Symposium on the Thymus, and, with its contributions by 72 authors, collects together

most of the groundwork on which our present knowledge is based. Of the 36 contributions, most are concerned with the relation of the thymus and bursa of Fabricius with lymphocytopoiesis and the development of immune responsiveness. Much of this material has been published elsewhere, and some has been reviewed also. Thus, it is a pity that this book was not published more promptly after the symposium (held in 1962) when it might have had greater value.

Many of the old problems still remain: there is still no convincing evidence that the thymus, either directly or via the lymphocyte, has any trophic function on other tissues; and there is still no convincing way of determining whether thymic lymphocytes have an epidermal or mesodermal origin. However, the distinct functions of the thymus and bursa in the chicken become clearer now that thymectomy has been achieved while leaving the bursa intact.

There are lighter moments: as when one author presents the same unconvincing experiment which he first described in 1953. Another author, starting with an *idée fixe* that the thymus is concerned with growth, is continually frustrated by finding that each of his 'thymic' growth factors is a general tissue constituent. The clinical papers, though often long-winded, are perhaps the most valuable, since much of this material is not always readily accessible. Here is a fascinating collection of facts which will correct any tendency to over-simplify from the results of pure research.

There are many illustrations, which are mostly good, although the finer points of lymphoid tissue structure are not well conveyed by the usual black-and-white print. While the book will be of value in some libraries, there may be few individuals to whom it is worth the high cost.

R. B. Taylor

Advances in Enzyme Regulation

Vol. 2. Edited by George Weber. (Proceedings of the Second Symposium on Regulation of Enzyme Activity and Syntheses in Normal and Neoplastic Liver, held at Indiana University School of Medicine, Indianapolis, Indiana, September 30 and October 1, 1963.) Pp. xii + 405. (London and New York: Pergamon Press, 1964.) 100*s*. net.

THE symposia on "Regulation of Enzyme Activity and Syntheses" seem now to be established annual events, and the proceedings of the second meeting show that enzyme regulation in mammalian systems is nowadays the subject of as much research as is that of micro-organisms.

The volume, like the symposium, is arranged in six sections, each under a distinguished chairman. The first, on the regulation of gluconeogenesis, contains valuable discussions on the roles of the enzymes pyruvate carboxylase, phospho-enol-pyruvate carboxylase and fructose diphosphatase in gluconeogenesis and suggests an action of insulin as a repressor of some essential enzymes of this pathway. The second and third sections remain centred on carbohydrate metabolism and present data on acetyl–coenzyme *A* metabolism, the role of some steroids in gluconeogenesis, human hypoglycemia and the regulation of glucokinase. The synthesis of this last enzyme is inducible, being stimulated by glucose and by insulin.

Section four, on feed-back regulation in hepatic systems, contains papers on the enzymes lactic dehydrogenase, aspartic transcarbamylase and phosphofructokinase, on glucose regulation of enzyme synthesis and on regulation of cholesterol biosynthesis. Section five includes discussion of the substrate and coenzyme-stimulated induction of tyrosine transaminase; the final section, on enzyme regulation in hepatomata, presents studies of the variation of enzyme activities in tumours of different characteristics. Some correlation between enzyme activities, metabolic patterns and growth rates is analysed.

The volume is attractively presented, and represents a valuable progress report on a very active field of research.

C. J. R. Thorne

Seed-borne Fungi

Description of 77 Fungus Species. By J. P. Malone and A. E. Muskett. (Proceedings of the International Seed Testing Association, Vol. 29, No. 2.) Pp. 179–384. (Wageningen: Association Internationale d'Essais de Semences, 1964.) n.p.

THE authors of *Seed-borne Fungi* are well known for their experience in seed testing, and theirs is a compact and strictly practical production. After a brief historical introduction, the book contains a description of 77 species of seed-borne fungi, each illustrated by clear photographs of growth habit, fructification and/or spores, as may be appropriate to the species. The approved method of testing for its presence is indicated under each. Most of the fungi found on germinating seeds are harmless saprophytes but it is important that the tester shall be able to recognize these for what they are, and they are accordingly figured as carefully as the known parasites. The latter include 5 smuts (one, however, a common contaminant from cereal weeds) and 14 ascomycetes, or conidial states presumed to belong to ascomycetes. The 57 species in 36 genera of saprophytes described include 4 genera of ascomycetes which normally produce perithecia under test conditions, including 11 species of *Chaetomium*, 2 of *Melanospora* and 2 of *Sordaria*, all clearly figured, as well as 4 genera of phycomycetes. The fungi are arranged alphabetically, irrespective of their systematic position, often under the name of the ascus state even though only the conidial state is to be expected to appear under the test conditions. This has the awkward effect of dispersing the species of *Fusarium* described under *Fusarium*, *Gibberella* and *Griphosphaeria*, while those of *Helminthosporium* have to be sought under that name and under *Cochliobolus* and *Pyrenophora*. The treatment is not consistent, for *Botrytis cinerea* appears under that name, not as *Sclerotinia fuckeliana*, nor does *Cladosporium herbarum* masquerade under *Mycosphaerella tulasnei*, though to find *Stemphylium botryosum* we must look under *Pleospora herbarum*. Such difficulties as may arise from this treatment are resolved by an adequate index, but if one has to find a species through the index after all, the advantage of an alphabetical arrangement over a taxonomic one is lost. One feels that a taxonomic arrangement based on conidial states where they exist would be more helpful to an inexperienced worker seeking to name an unfamiliar species.

R. W. G. Dennis

Methods for the Examination of Root Systems and Roots

By Dr. J. J. Schuurman and Dr. M. A. J. Goedewaagen. Pp. 86. (Wageningen: Centre for Agricultural Publications and Documentation, 1964.) 10.50 D.fl.; 21*s*.; 3 dollars.

THE underground parts of plants have been consistently neglected by botanists, most of whom have little idea how to set about investigating the morphology of a root system. This little book is therefore to be welcomed, for it describes concisely the various methods of investigation which have been used, those favoured by the authors being supported by numerous practical hints. The methods covered include the transferring of a soil monolith to a pinboard, the mapping of a profile face, the sampling of root systems by means of auger borings, and the growing of experimental plants in special containers. The scope of the book is confined to methods, and the sceptical are left to wonder whether the arduous task of disinterring roots yields a commensurate return of information. Production of the book is good; but the reader will encounter a few eccentricities of vocabulary, such as pendulum for crank handle.

W. D. Clayton

THE SMITHSONIAN INSTITUTION, WASHINGTON, D.C.

A COLOURFUL academic procession opened the three-day (September 16–18) celebration, marking the two-hundredth anniversary of the birth of James Smithson, founder of the Smithsonian Institution, Washington, D.C. Prof. S. Dillon Ripley, the present chief executive and secretary of the Institution, said that the bicentennial paid tribute to the Institution's past, affirmed its intent to fulfil a vital role in society, and examined the nature of knowledge and creative discovery as conceived by James Smithson and as understood to-day.

On September 16 the procession, which began with a clarion call from a tower of the world-famous red Smithsonian 'castle', included some 500 world scholars and scientists. Colonel John H. Magruder, of the U.S. Marine Corps, acted as the grand marshal, leading the distinguished gathering from the original Smithsonian Institution building across the Washington Mall, to a site near the Museum of Natural History.

Here, the President of the United States addressed the delegates in an outdoor convocation. Dr. Leonard C. Carmichael, former secretary of the Smithsonian, also gave an address, the substance of which follows.

Smithson, an English scholar, scientist, and prominent member of the Royal Society of London, left his entire estate to the United States, a country he had never seen, "to found at Washington an establishment for the increase and diffusion of knowledge among men". He died in Genoa, Italy, in 1829.

To-day, his 'legacy' comprises twelve far-reaching units:

(1) The Museum of Natural History, which has separate offices of anthropology, ecology and systematics, and departments of vertebrate and invertebrate zoology, entomology, botany, palaeobiology, and mineral sciences, and an oceanography programme.

(2) The Museum of History and Technology, which has departments of science and technology, arts and manufactures, civil history, and armed forces history.

(3) The National Air Museum, containing the world's largest collection of historical aviation and aerospace material.

(4) The Freer Gallery of Art, specializing in near and far eastern art.

(5) The National Collection of Fine Arts.

(6) The new National Portrait Gallery, which will feature portraits and statues of men and women who have made significant contributions to the history, development and culture of the United States. This gallery will not be fully developed until 1967.

(7) The National Zoological Park.

(8) The International Exchange Service, which exchanges scientific, cultural, literary, and governmental publications with other countries.

(9) The Science Information Exchange, which receives, organizes and disseminates information about scientific research in progress in the United States.

(10) The Astrophysical Observatory, located at Cambridge, Massachusetts.

(11) The Canal Zone Biological Area, a biological preserve located on Barro Colorado Island in Gatun Lake, Canal Zone.

(12) The National Gallery of Art, which has a separate Board of Trustees.

The John F. Kennedy Center for the Performing Arts, to be built soon in Washington, is also a bureau of the Smithsonian Institution, administered separately by its own Board of Trustees.

Fig. 1. The Smithsonian Institution

JAMES SMITHSON: PATHFINDER IN SCIENCE AND PHILANTHROPY

By Dr. LEONARD CARMICHAEL

Vice-President for Research and Exploration, U.S. National Geographic Society, and Former Secretary of the Smithsonian Institution

"Why did he do that?" Her Majesty Queen Elizabeth, the Queen Mother, asked me. I was walking with her as I showed her the Museum displays of the Smithsonian Institution. I had just said that James Smithson, the Institution's founder, was an English philanthropist who had never been on the American continent, but when he died he left his whole large fortune to found the Smithsonian in Washington.

"Why did he do that?" I had to reply that no one knew. But, as a partial answer, I would like to say something of the life of this idealistic lonely English scientist whose bequest has done so much for America and for the world and whose birth 200 years ago is now being honoured.

The basic facts of the life of this benefactor are sad and hard. H. R. Tedder, his English biographer, says he was "a man of gentle character whose life was devoted to study uncheered by domestic affection". He was the natural son of Sir Hugh Percy, first Duke of Northumberland of the third creation. This nobleman, in spite of a reputation for arrogance, was one of the great figures of his day. A contemporary called him the handsomest man in England. He had a large personal fortune and he held many posts of honour. He served as a Privy Councillor and Lord-Lieutenant of Ireland. His own family name was Smithson before it was changed to that of Percy by Parliament.

James Smithson's mother was a widowed heiress, Mrs. Elizabeth Hungerford Keate Macie, who was herself descended from King Henry VII through the line of the Dukes of Somerset. Much of his mother's considerable wealth came from her own family, the Hungerfords, and from the estate of her deceased husband, John Macie, a country gentleman of Bath, Somerset. Her son, James, was born in France in 1765, and for this reason it was later necessary for him to be naturalized as an English subject, even though both his parents were English. Strangely, when this petition was granted by the Crown, it contained unusual provisos. It granted James the advantage of being a British subject except for certain stipulations such as that he was never to be allowed to be a member of the Privy Council or of Parliament or to be a civil or military officer. Neither could he receive any hereditaments from the Crown. Thus, unlike many scientists of his day and ours, he was never knighted.

J. C. Long, who has recently written a new life of Smithson based on extensive research in England and to whose scholarship I owe much in preparing this address, points out that these restrictions in his grant of naturalization were most extraordinary. He feels that they must have been inserted by someone with power in court circles who wished to guard against a possible political challenge later in life by this personable, wealthy, and well-born young man. Since the Duke had been a Privy Councillor, the specific mention of this office as barred to his natural son may not be without significance in hinting at the author of the surprising prohibitions. Many facts about Smithson's life are in doubt and may always remain so, because his personal papers, which were sent to America when his Institution was founded, were lost in the disastrous Smithsonian fire of 1865. Two hundred of Smithson's unpublished manuscripts and thousands of items of his personal memorabilia and his mineral collection of some ten thousand specimens perished at that time.

Fig. 2. Museum of History and Technology, Smithsonian Institution

In 1782, at the age of seventeen, James matriculated at Pembroke College, Oxford. Here, because of his family, he wore the special gown and had the privileges of a 'gentleman commoner'. On matriculation he signed the thirty-nine articles of the Church of England, and throughout his life he remained loyal and constant in his membership in this church. This was true even during the extensive periods of his life which were spent on the Continent of Europe.

James mastered well the regular classical studies of his period at Oxford. Almost from the first, however, he became interested in the then extra-curricular activity of the study of experimental science. Soon he was recognized as one of the leading authorities at Oxford in the infant science of chemistry. He also became an intensive student of geology and mineralogy. While still an undergraduate he was a scientific member of an important and well-organized geological expedition under the direction of a distinguished French mineralogist.

At twenty-one years of age, in 1786, he graduated with the degree of master of arts. His already achieved distinction as a chemist is attested by the fact that eleven months after he received his degree he was elected a Fellow of the Royal Society. He seems to be the youngest man ever to have received this honour. The Royal Society, then as now, was recognized as having no peer among the learned academies of the world. It is indeed amazing to think of a scientist of twenty-two being able to write after his name the great letters, F.R.S.

He soon established himself as an almost-official helper and close colleague of Henry Cavendish, the discoverer of hydrogen and one of the most distinguished scientists of the eighteenth century. This scholarly man, son of Lord Charles Cavendish and grandson of the Duke of Devonshire, was the possessor of a large personal fortune, but he elected to live almost a hermit's life and to develop the excellent private laboratory in which Smithson worked with so much satisfaction.

James Macie, as Smithson was known at that time, also established close friendships with other scientists in London and especially with Sir Joseph Banks, president of the Royal Society. Other friends were Sir Humphry Davy and the American-born Benjamin Thompson, Count Rumford.

Before his mother's death she expressed the wish that her son adopt the name Smithson, his father's family name. As I have noted, his father had taken the name Percy when he was created Duke of Northumberland. It is interesting that Smithson's mother and also Sir Hugh's wife were related by blood to the Percy family, but the Duke himself was not. In 1801 the petition for his change of name was granted, and thereafter the founder of the Institution is officially James Smithson.

As a young scientist Smithson was assiduous in attending meetings of the Royal Society and was active in his own programme of experimental research. He soon began the publication of important scientific papers. To-day we hear of the so-called 'publish or perish' policy of some large universities. Smithson would have passed this test with honour. In all he published some twenty-seven papers in the most respected scientific research journals of his day.

Smithson never married, and in spite of the gay world into which he was born and his ample fortune he devoted his life to study and research. Not only was he active in scientific associations but he also visited and worked with leading research men of his day in Berlin, Paris, Rome, Florence, Genoa, and Geneva. He knew France well under the monarchy, but when anti-Royalist sentiments became common in Paris he found himself sympathetic with them. Indeed, all his life he was philosophically favourable to a republican form of government rather than to a monarchistic one, but he deeply disapproved of the excesses of the French Revolution. He did, however, understand its basis in the errors of the old régime. He wrote in this connexion: "Stupidity and guilt have had a long reign and it begins indeed to be the time for justice and common sense to have their term".

No one knows how close Smithson was to his autocratic, handsome, and politically powerful father, the Duke of Northumberland, or to the Duke's other children who were his half-brothers and -sisters. It is interesting that one of his half-brothers, Earl Percy, who later succeeded his father as Duke of Northumberland, was a Lieutenant-General of the British forces during the American Revolution. He led British troops back from the battle of Lexington and Concord. He secured his recall from America because he had long disapproved of the policy of George III towards the colonies. General Percy, indeed like James Smithson and some other members of his family, was a very moderate Tory and much attracted to republican theories. He was known as an admirable landlord and as a most generous officer by those under his command. How much these attitudes and especially his view of America influenced his younger half-brother, James, we probably will never know.

James Smithson was disturbed by the success of Napoleon in Europe, but he continued to visit the Continent for study and research. In 1807, he was seized as a prisoner of war by the Napoleonic authorities as he was about to sail back to England from Denmark. His treatment as a prisoner was harsh, and he never fully recovered from the chest illness which he acquired at this time. Almost certainly as a result of the intervention of his friend Sir Joseph Banks of the Royal Society, the King of Denmark interceded on behalf of Smithson. Even with this help, although his lot was brightened, he did not succeed in returning to England until Napoleon's power waned after the disastrous Russian campaign of 1812.

In 1800 and 1801, before these difficult prison years, Smithson had been one of the group which established in London the Royal Institution. This new organization had and has a remarkable mission which in many ways suggests the purposes of Smithson's own later institution in Washington. The purpose of the Royal Institution as set forth in its charter is:

> "For diffusing the knowledge and facilitating the general introduction of useful mechanical inventions . . . and for teaching by courses of philosophical lectures and experiments the application of science to the common purposes of life."

As I have already stated, most of Smithson's own scientific work dealt with chemistry or mineralogy and with what would now be called applied science. One of his most notable contributions was his development by the ingenious use of the still-primitive chemical methods available of the basic distinction between the native carbonates and silicates of zinc. As a result of this work the beautiful and economically important zinc carbonate ore is now called 'smithsonite'. One of Smithson's real memorials at his Institution in Washington is a specially constructed case in which various forms of brilliantly coloured smithsonite slowly revolve on a turntable so that each year hundreds of thousands of visitors to the Institution's Hall of Minerals and Gems can see the full beauty of the founder's great mineral.

Smithson also was one of the pioneers in what is to-day called microchemistry. He was able to analyse quite minute quantities. On one occasion he effectively identified the salts in solution of half a drop secretion from a human lacrimal gland. He also developed a method for the detection of very minute quantities of arsenic and mercury.

In spite of his concern with experimentation he was also interested in the theory and philosophy of science. One of his papers deals in an effective way with the errors of a contemporary scientist who tried to see unjustifiable biblical significance in fossil bones collected in a Yorkshire cave.

Smithson was well aware of the insufficiency of scientific knowledge of his time. He said: "What we know in

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chemistry bears so small a proportion to what we are ignorant of; our knowledge in every department of it is so incomplete, consisting so entirely of isolated points, thinly scattered, like lurid specks on a vast field of darkness, that no researches can be undertaken without producing some facts leading to consequences which extend beyond the boundaries of their immediate object".

He was also far ahead of his time in concerning himself with the fundamentals of scientific research and scientific truth. At one point he wrote in adumbration of much later knowledge: "The particle and the planet are subject to the same laws, and what is learned of one will be known of the other".

The eighteenth century was not a period noted for great personal morality on the part of many members of its wealthy and influential families; but no breath of scandal, even by surmise, has attached itself to James Smithson. It may be noted, however, that he did have one form of recreation that may call for the raising of at least some proper modern eyebrows. He liked to gamble. He was well known in the casinos of European capitals. But Smithson was a good mathematician, and he early convinced himself that the only consistent winner in a gambling hall was the proprietor. He even calculated with care what such a man's winnings were apt to be. Because of this knowledge he always limited his expenditures at the table of chance to a predetermined sum which was well within his means for an evening's entertainment.

Three years before his death Smithson wrote his famous Will. It began as follows: "I James Smithson Son to Hugh, first Duke of Northumberland, and Elizabeth, Heiress of the Hungerfords of Studley, and Niece to Charles the proud Duke of Somerset, . . make this my last Will and Testament. . . ". The Will provided that the income of his estate should go to his nephew, and if the nephew later had children the principal should go to them. But if his nephew should die without heirs he bequeathed the whole of his property, "to the United States of America to found at Washington under the name of the Smithsonian Institution an establishment for the increase and diffusion of knowledge among men".

On June 26, 1829, Smithson died in Genoa, Italy. In this city he had close scientific colleagues. He had been a devout attendant at the Church of England services in that city, and it was after services in this church that he was buried in the Protestant cemetery of that city. To-day there is in the English Church of the Holy Ghost at Genoa a fine bas-relief monument to Smithson erected by the Regents of the Smithsonian Institution.

His nephew died without heir on June 5, 1835, and seven weeks later President Andrew Jackson in an address to Congress made formal recognition of the gift to the American nation.

After prolonged discussion and largely as a result of the effective work of John Quincy Adams, who had returned to the House of Representatives after his period as President of the United States, an act authorizing the Smithsonian Institution was passed by the Congress and signed by President Polk on August 10, 1846. The English gold sovereigns in which the bequest was paid were brought to America and recoined at the Philadelphia Mint. In new American money the bequest was 508,418·46 dollars.

The bequest may not seem very great to-day, but at that time it was correctly recognized as a most significant sum. No comparable amount of money then existed in America in any university or other establishment to be used as an endowment to support research or the diffusion of knowledge.

Under the administration of the first Smithsonian secretary, the distinguished scientist Joseph Henry, and with the support of an able Board of Regents, the Institution was soon actively engaged in its work of fostering new scientific research. Through the years Smithson's phrase, "the diffusion of knowledge", has been interpreted at his Institution to mean the issuing of books and monographs and the development of truly instructive museum displays. At least 12,000 separate publications have been issued by the Smithsonian Institution.

At about the turn of the century word was received that land changes were threatening the area of the cemetery in which Smithson was buried in Genoa. The Regents of the Smithsonian delegated their very distinguished member, the great telephone inventor, Alexander Graham Bell, to go to Genoa and investigate the condition of the founder's grave. As a result it was decided that Smithson's remains should be brought to Washington. His marble sarcophagus was transported with honour to the United States, and borne from the Washington Navy Yard to the Institution, accompanied by a troop of cavalry, a detachment of Marines, and the Marine Band. To-day, under English and American flags, the sarcophagus rests in a beautiful chapel in the Institution's original building on the Mall here in Washington.

So, as we think of Smithson's quiet and useful years and of his amazing gift to the United States, we can again consider the Queen Mother's query: "Why did he do that ?". Still it must be said no one can answer this question with assurance, but I hope some people may feel with me that an understanding of Smithson's life makes it apparent that he was a prudent but true son of the 'Enlightenment'. In youthful and active America he saw much that was hopeful for the human race everywhere. His own Institution has certainly been true to his ringing charge, and in each year since its founding it has transformed some good aspect of his dream into solid serviceable reality.

When the Smithsonian was established, Washington was a swampy and fever-ridden community of some 5,000 people, and the country of which it was the capital had scarcely begun the kind of scientific research which even then was providing much humanly important, pure and applied knowledge in the older European countries. The careful handling of the income from Smithson's bequest and of funds provided by other gifts, and above all by appropriations of the Congress of the United States, has allowed the Smithsonian Institution through the years to serve as a pioneer in the establishment of the great scientific tradition of the United States. To-day and indeed through all its years, the success of the Smithsonian has been due in large part to the devotion of its always-eminent and always diligent Board of Regents.

The utterly unselfish generosity and the broad and wise terms of Smithson's bequest aroused great interest in the United States and indeed in the world when it became known. In many ways this unprecedented gift still helps set a pattern for charitable and educational benefactions. Histories of the development of the great philanthropic foundations of the United States which are doing and have done so much for the world rightly begin with a reference to James Smithson's beneficence.

As a man Smithson was a loyal son of England. But as a scientist his horizon always was global. He once wrote: "The man of science is of no country, the world is his country, all mankind his countrymen". Surely those of us who have been privileged to know Smithson's Institution intimately cannot help feeling that by his gift he established an Institution which certainly in the future, as in the past, will continue the great work of the increase and diffusion of knowledge in a way that will benefit all mankind everywhere.

It is well that the following words of Smithson himself are cut in the stone of the Institution's great new Museum of History and Technology:

"Every man is a valuable member of society who, by his observations, researches, and experiments, procures knowledge for men . . . it is in his knowledge that man has found his greatness and his happiness, the high superiority which he holds over the other animals who inhabit the earth with him, and consequently no ignorance is probably without loss to him, no error without evil. . . ."

Such a "valuable member of society" indeed was James Smithson, the sagacious, far-sighted and truly philanthropic founder of the now honoured and superlatively useful Smithsonian Institution.

SCHOLARLY SESSIONS AT THE SMITHSONIAN BICENTENNIAL CELEBRATIONS

The following sessions were held during September 17–18. The chairman is named (in brackets) followed by the speakers.

(1) *Man's Origin and Progress* (Dr. Caryl P. Haskins, president of the Carnegie Institution and regent of the Smithsonian Institution): Prof. F. L. Whipple, director of the Smithsonian Astrophysical Observatory; Prof. Claude Levi-Strauss, director of the Institute of Ethnology, University of Paris.

(2) *Environmental Influences on Man* (Dr. Crawford H. Greenewalt, chairman of the Board, E. I. du Pont de Nemours and Co., and regent of the Smithsonian Institution): Dr. Ian McTaggart Cowan, dean of the faculty of graduate studies, University of British Columbia; Prof. J. Robert Oppenheimer, professor of physics and director of the Institute of Advanced Study, Princeton, formerly director of the Los Alamos Scientific Laboratory.

(3) *Man and Technology* (The Hon. A. M. Burden, former American Ambassador to Belgium and regent of the Smithsonian Institution): Prof. Herbert Butterfield, master of Peterhouse and professor of modern history in the University of Cambridge; Dr. Lewis Mumford, president of the American Academy of Arts and Letters.

(4) *Inheritance, Values and Creativity* (The Hon. John Nicholas Brown, former assistant secretary of the U.S. Navy for Air and regent of the Smithsonian Institution): Prof. G. Evelyn Hutchinson, Sterling professor of zoology, Yale University; Sir Kenneth Clark, former director of the National Gallery, London; Mr. Arthur Koestler, author.

(5) *Man's Developing Intellect and Attitudes* (Mr. George H. Mahon, United States Representative (Texas) and regent of the Smithsonian Institution): Prof. Jerome S. Bruner, professor of psychology and director of the Center for Cognitive Studies, Harvard University; Prof. Stephen E. Toulmin, professor of the history of ideas and philosophy, Brandeis University, former director of the Nuffield Foundation Unit for the History of Ideas, London.

JAMES SMITHSON MEDAL: ROYAL SOCIETY OF LONDON

The James Smithson Medal, instituted in memory of the British founder of the Smithsonian Institution, is an award for distinguished contributions to "the increase and diffusion of knowledge among men", the role of the Institution as set forth by James Smithson in making the bequest of his fortune to the United States.

The Medal is the creation of Paul Vincze of London, following closely a medallion made in 1817 by the well-known sculptor and engraver at the Paris Mint, Nicholas Pierre Tiolier, which was marked on the back in Smithson's own hand as "my likeness". Vincze has created on the obverse a portrait of James Smithson, surrounded by the name and dates of his birth and death, 1765–1829. The reverse bears a rendering of the familiar outline of the first Smithsonian building, for which the cornerstone was laid on the Mall on May 1, 1847.

The Medal was presented for the first time on September 18, 1965, to the Royal Society of London, during the recent bicentennial celebration. In the future it will be presented for exceptional achievement in the various disciplines of science and the humanities embraced by the Smithsonian Institution.

The first Smithson Medal for outstanding contributions in the fields of science, technology, history, and art was presented by Robert V. Fleming, chairman of the Board of Riggs National Bank and also chairman of the Executive Committee of the Board of Regents of the Smithsonian Institution.

EVOLUTION IN THE BIOSPHERE

By Prof. HAROLD F. BLUM*

National Cancer Institute, National Institutes of Health, Bethesda, and Department of Biology, Princeton University

EVOLUTION in the biosphere—whether biological or cultural—is based on the persistence of patterns which are on occasion subject to alteration, the altered pattern persisting in turn until another alteration occurs. The genotype pattern of a species persists through replication in the germ cells from generation to generation, subject to rare modification which constitutes mutation. Cultural patterns are also perpetuated; we do not know much about the mechanism in this case; but it is clear that they may persist for long periods although readily modifiable when circumstances arise[1].

Perpetuation makes possible the selection of some patterns—according to the dictates of environment or other factors—with the consequent rejection of others, for example, the natural selection of a mutant form from a number of possible alternatives. By restricting our view to the selected patterns and disregarding the rejections we see evolution as an increase in order, although the order is related inversely to the number of rejections. We may picture the increase in order in the evolving system as accompanying the addition of facets of pattern to the pattern as a whole; somewhat analogous to increase in order with growth of a crystal. Since change in pattern is relative to existing pattern, the number of facets may be expected to grow exponentially; thus increase in order is accompanied by limitation of subsequent change to a general type of pattern, but at the same time the possibilities of change within the scope of that pattern may increase.

Analogy may be made to a computer, where the number of possible answers is decreased by selection of one answer instead of another, each question being postulated on the previous one[2]. The increase in order of the evolving system should, then, be expressible in a form similar to that applied to computer operation:

$$-\Delta s = q \ln \frac{f_2}{f_1} \qquad (1)$$

where $-\Delta s$ is the negentropy change, f is the number of facets of biological or cultural pattern in the evolving system, q is a constant, and ln indicates the natural logarithm. Successful mutations and adopted cultural innovations represent additions of facets to respective existing patterns.

Perpetuation of both genetic and cultural patterns depends on perpetuation of the kinds of organisms which carry them. This involves maintenance as well as reproduction, paid for in terms of free energy amounting to about 2×10^{18} kg cal/yr captured from sunlight by photochemical reduction of carbon dioxide. The fraction of the total free energy expenditure which supports the

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selection of changes in pattern directly concerned in the increase of negentropy must be extremely small, so no evasion of the second law of thermodynamics need be entailed in the increase of order which accompanies evolution.

Although the supply of free energy limits the rate of accumulation of negentropy, it does not control the direction which evolution may take, any more than the amount of electrical energy supplied to a computer influences the answers it gives to questions. Thus, although the biosphere is ultimately dependent on the Sun for its supply of energy, the course of evolution may be treated as virtually independent of events in, and properties of, the universe beyond Earth's limits. Some influence from outside our planet must, of course, be admitted—the rate of occurrence of mutations may be influenced by ionizing radiation from external sources, and investigation of the heavens has played a profound part in Man's cultural evolution—but we may neglect these as minor factors in our view of the process as a whole.

Evolution in the biosphere does not constitute movement toward greater order *per se*; but is guided by changes in specific properties, as contrasted with changes in number of arrangements of properties in terms of which change in order is measured, and these two things need not run closely parallel. (May we not think of free energy as depending on specific arrangements of microscopic properties, as contrasted to the number of arrangements of microscopic properties in terms of which entropy is measured ?) For example, natural selection of a mutant over a parent form depends on specific properties of the two phenotypes and on specific properties of the environment with which they impinge—the resulting change in the genotype pattern entails, but does not depend on, a change in order.

Whether dealing with computer operation or with evolution, the assumption is usually tacit that increase in order is accompanied by increase in meaning[2], which must presumably be defined in terms of specific properties. Even though we might conceive of measuring meaning in terms of all the specific properties of the system, we would still not know how to fit our measurements into the same dimensional framework with the number of facets of orderly pattern. In practice we assess meaning in terms of subjective judgments (although sometimes this is concealed), thus substituting attributed meaning for the true meaning we cannot hope to measure; this would seem a potential source of error in thinking about the evolution of living systems.

It may be useful to formalize this situation by assuming quantitative relationship between attributed meaning, true meaning, and orderly arrangement of facets, representing this by:

$$x' \ln \frac{m'_2}{m'_1} = x \ln \frac{m_2}{m_1} = q \ln \frac{f_2}{f_1} \qquad (2)$$

where m is a quantitative measure of true meaning, m' a measure of attributed meaning, and x and x' are respective constants which we imagine can be related to each other and to q within a common dimensional framework, although knowing that this cannot really be done.

Exponential growth of each of the variables, m', m and f, expressed in equation (2), may be rationalized on the basis that change in pattern is always relative to existing pattern, whether involving change in number of facets or specific change in properties, and that our subjective estimates presumably follow the latter in a general way. Thus, in whatever terms measured, the addition of each facet increases the possibility of expansion of the pattern. We may, for example, expect culture to evolve exponentially because usually each cultural innovation increases the chance of further innovations[1]; even when there may be loss of facets, this should be relative to the existing pattern.

Evolution of the biosphere may be thought of as composed of many interplaying parts, and equation (2) may be applied to the whole or to a single one. In practice, our attention is usually focused on a particular part, say the species of a given genus or a single aspect of culture, which it is convenient to treat as a separate system independently of the whole. In so doing, however, we should not lose sight of the possible effects of other parts on the one we study, but we cannot expect to fit x' and x for different parts into the same dimensional framework.

The changes in specific properties which may be regarded as steps in evolution—successful mutations, and cultural innovations—may be thought of as chance events distributed successively in time. But since any given step depends on an existing pattern, the probability of the step occurring must be relative to the existing pattern, and hence not to be treated as a strictly random happening. Thus, the rate of evolution cannot be regarded as completely independent of the pattern of the evolving system, but for purposes of description we may write:

$$x' \ln \frac{m'_2}{m'_1} = \int_{t_1}^{t_2} f(t)\mathrm{d}t \qquad (3)$$

where $f(t)$ is a function which may take a variety of shapes; the equation is written for attributed meaning since it is this we perforce investigate.

In the simplest form equation (3) might take, $f(t)$ would be a constant, α:

$$x' \ln \frac{m'_2}{m'_1} = \alpha t \qquad (4)$$

Equation (4) should be thought to hold in only a statistical sense, since it is unlikely that all evolutionary steps can be given equal weight or that they occur at regular intervals of time, as the equation might imply. Such simple exponential growth seems fairly closely followed, nevertheless, in some instances, by various aspects of cultural evolution for short periods of time[3], and by number of species of molluscs[4]. But it would seem impossible that a curve of this form could be followed indefinitely, evolution being eventually restricted for various reasons; for example, increasing adaptation to an ecological niche would seem to impose restrictions on evolution of the given species, development of one aspect of culture may place restrictions on another, and the size of the system must itself impose limits.

If we assume that the probability of selecting new facets of pattern falls off exponentially with time due to such restriction, we may write as a general expression:

$$x' \ln \frac{m'_2}{m'_1} = \beta(1 - \mathrm{e}^{-\gamma t}) \qquad (5)$$

where β and γ are constants, and e indicates the base of natural logarithms.

Curves described by equation (4) may represent early parts of curves described by equation (5), since for small values of t the function $(1-\mathrm{e}^{-\gamma t})$ is very nearly equal to γt. On the other hand, for large values of t the function approaches unity, thus describing approach to a static condition, as may be exemplified in the brachiopods and in *Sphenodon*, where there has been little evolution for very long periods of time. In a preliminary search I have not found many data on biological evolution which seem appropriate for such analysis. On the other hand, aspects of cultural evolution often indicate relative growth; some curves approach a limit after the manner of equation (5), others follow equation (4) more or less closely, but sometimes show fluctuations which suggest acceleration and deceleration of relative growth. It is proposed to discuss elsewhere a variety of curves describing aspects of cultural evolution, among which there may seem little similarity at first glance; but which may be reasonably well fitted by equation (3) when simple, logical forms are

assigned to $f(t)$. All this suggests that evolution follows variations on an exponential theme, and may be thought of as a relative growth process.

Let us imagine, for purposes of contrast, an environment with a fixed number of ecological niches each one of which can best be filled by a species having a specific inherited pattern. Then, given adequate time, all species with inherited patterns that do not fit best will be eliminated, leaving every niche perfectly filled. In this kind of panglossian world, where everything happens for the best if one waits long enough, evolution might be expected to follow a more or less linear course, and to have only one ultimate outcome. One wonders at times whether some such a world is not tacitly assumed in a good deal of present thinking[5]. Recent achievements in biochemistry indicating nice evolutionary series of molecular structure may have encouraged this thinking[6]: but such series are, of course, to be expected if evolution follows a course of relative growth.

If one adopts the idea that each chance step in evolution can only be taken in terms of an existing pattern—which seems imposed by modern concepts of genetics—one admits the possibility that evolution in another biosphere than ours might, although starting from nearly identical initial conditions, arrive at an array of species and cultures widely different from those we know, or even none whatsoever. Only by tacitly disregarding the possible alternate pathways open each time a facet of pattern was selected[2] does one arrive at the notion of inevitableness in evolution; yet selection from among alternate pathways is the essence of Darwinian theory.

I thank Prof. Roger S. Pinkham for his advice.

Note added in proof. After writing this article, one by Sir Karl Popper has appeared (*Nature*, 207, 233; 1965) which has been taken by at least one reader to suggest that evolution in the biosphere runs counter to the second law of thermodynamics. (My book, *Time's Arrow and Evolution*, Princeton Univ. Press, 1951, revised printing, Harpers, 1962, is wrongly cited there, which may be confusing to a reader wishing to examine the original statements referred to.) In both this and another recent paper (*Nature*, **206**, 131; 1965) it is pointed out that a thermodynamic balance sheet cannot be drawn up for evolution in the biosphere, because the dimensions of the system cannot be properly evaluated; but there seems no reason to assume that the second law is disobeyed.

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[4] Cailleux, A., *C. R. Soc. Geol. France*, 222 (1950).
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NEWS and VIEWS

Pacific Science Center Foundation Arches of Science Award : Dr. Warren Weaver

Dr. Warren Weaver has been elected to receive the Arches of Science Award of the Pacific Science Center Foundation. This comprises a cash prize of 25,000 dollars and a gold medal. It will be presented to Dr. Weaver at the Pacific Science Center on October 25. The Arches of Science Award is presented to an American who has made "the outstanding contribution to the public understanding of the meaning of science to contemporary man". The first recipient of the Award, Dr. Weaver is well known as a 'communicator of science', and is recognized as one of the outstanding public interpreters of the scientific revolution of the past half-century. Associated with the Rockefeller and Sloan Foundations for more than thirty years, he is also an internationally known mathematician, administrator of some of the leading scientific research institutions in the United States, an author, editor and lecturer. In announcing the Award, Dr. Edward E. Carlson, president of the Pacific Science Center Foundation, said: "Dr. Weaver's selection for this new Arches of Science Award adds prestige comparable to the highest prizes for science achievement. The scientific community, because of their regard for Warren Weaver, is characterizing the Award as an American Nobel Prize—and it is significant that more than fifteen Nobel Laureates in science have received Dr. Weaver's early recognition and support".

During his response to the announcement of the Award, Dr. Weaver said: "Most of the previous significant rewards for scientists have been given exclusively for what the individual has done inside of science, strictly for notable contributions to scientific research. Individuals who can make such contributions clearly deserve society's highest honours. But the relations of science to society, to our total culture, and incidentally to our government, are now quite unlike what they were even twenty-five years ago. It is essential that we to-day have individuals who are capable of understanding science, and who are willing to live their lives partly within science but also partly within the world of affairs. These persons, working at the interface of science and society, are more than useful—they have become essential". Sponsor of the Award is the Pacific Science Center Foundation of Seattle, Washington. The Foundation is a non-profit agency composed of business, scientific and educational leaders in the Pacific north-west who have been joined by leading men of science throughout the nation to create an active and permanent centre dedicated to increasing the public understanding of science. The Foundation operates the Pacific Science Center which was the popular U.S. Science Exhibit at the Seattle World's Fair of 1962. The Arches of Science Award was named for the five instantly recognizable arches soaring into the sky above the Pacific Science Center. The arches have become symbols of the technological strides and superior climate for science and science understanding emerging from the Pacific north-west.

New Zealand Department of Scientific and Industrial Research : Dr. K. A. Wodzicki

Dr. K. A. Wodzicki, director of the Animal Ecology Division of the New Zealand Department of Scientific and Industrial Research, retired recently after holding the position for sixteen years. Dr. Wodzicki was born in Poland and educated at the University of Cracow where he completed his M.Sc. and Ph.D. degrees. After serving as a lecturer at this University he was later appointed professor of animal physiology at the University of Warsaw, where his research work was concerned mainly with the genetical and ecological aspects of birds and animals. At the outbreak of the Second World War he escaped to France where he joined the Polish Government in Exile, and in 1941 was appointed as its Consul-General in New Zealand. In 1946 he was employed by the Department of Scientific and Industrial Research to survey the wild-life problems in New Zealand, and following the preparation of his report—which was later issued as a Departmental *Bulletin*—it was decided to set up an Animal Ecology Section of the Department with Dr. Wodzicki as officer in charge. Under Dr. Wodzicki's direction the Section has undertaken basic research on the ecology and physiology of introduced small animals, such as rabbit, hare and the mustelid family, and of birds such as

rooks. Dr. Wodzicki made a special study of the gannets which nest at Cape Kidnappers, Hawke's Bay. This study is recognized by ornithologists as one of the most rewarding of its type carried out anywhere, and has produced a great deal of new information about this bird.

Dr. J. A. Gibb

Dr. J. A. Gibb, who will succeed Dr. Wodzicki as director of the Animal Ecology Division of the Department of Scientific and Industrial Research, was born in Dorset, England, and educated at Sherborne School and at the University of Oxford, where he gained his M.A. and D.Phil. in zoology for research work on bird and insect populations carried out at the Edward Grey Institute. During the Second World War he served with the Royal Artillery in the Mediterranean Sector. Dr. Gibb joined the New Zealand Department of Scientific and Industrial Research in October 1957 as an animal ecologist, and was put in charge of the Division's major research project in rabbit ecology concerned with a study of population dynamics and feeding behaviour. In 1963 Dr. Gibb visited the United Kingdom and the United States to attend a meeting of the British Ecological Society and to visit various research institutions; in 1964 he visited Australia to attend a conference on "Vermin Control".

Mr. E. W. Hullett

Mr. E. W. Hullett has retired as director of the Wheat Research Institute of the New Zealand Department of Scientific and Industrial Research. Mr. Hullett went to the University of Canterbury (New Zealand), where he gained his M.Sc. degree with honours in chemistry. He was later awarded a National Research fellowship for study overseas, and on return to New Zealand became a lecturer at Lincoln College before joining the staff of the Wheat Research Institute in 1934; in 1936 he was appointed chief cereal chemist, and in 1950 succeeded Dr. O. Frankel as director of the Institute. Mr. Hullett has specialized in cereal chemistry and its application to milling and baking techniques, and under his directorship the milling and baking industries have benefited greatly from the research undertaken and the testing and information services provided by the Institute.

Mr. R. W. Cawley

Mr. R. W. Cawley has been appointed director of the Wheat Research Institute in succession to Mr. E. W. Hullett. Mr. Cawley graduated M.Sc. with honours in chemistry from Auckland University College in 1948. After lecturing for two years at Massey University, Palmerston North, he was appointed to the staff of the Wheat Research Institute as assistant chemist in 1950. His research work has been concerned with cereal biochemistry, particularly with the process of dough fermentation. In 1963 he was granted a research fellowship at the Wheat Research Unit of the Commonwealth Scientific and Industrial Research Organization, Australia, where he made a special study of pentosans, and their role in bread-making.

Mr. N. Modriniak

Mr. N. Modriniak has retired from the position of superintendent of the Geophysical Survey, Geophysics Division, New Zealand Department of Scientific and Industrial Research. Mr. Modriniak, who was educated at Leoben, Austria, arrived in New Zealand in 1927 and was engaged on geophysical prospecting with private companies for the following six years. In 1933 he joined the New Zealand Department of Scientific and Industrial Research, and until the outbreak of the Second World War he was concerned mainly with prospecting alluvial deposits for gold. In this period he pioneered the use in New Zealand of seismic methods for the investigation of shallow structures. Mr. Modriniak was among the first to become interested in the possibility of deriving power from geothermal steam in New Zealand, and the exploration of geothermal resources led to a major expansion of the Geophysical Survey in the late 1940s. During the early 1950s, when interest in and optimism about New Zealand's oil resources were at a low ebb, Mr. Modriniak persevered with seismic reflexion tests in the major sedimentary basins with the view of stimulating the interest of the major oil companies. While geothermal power, engineering and oil have been the main concerns of Mr. Modriniak as a Government scientist, he has always been actively interested in the fundamental geophysical structure of New Zealand and the south-west Pacific region generally.

Dr. T. Hatherton, O.B.E.

Dr. T. Hatherton has been appointed to succeed Mr. N. Modriniak as superintendent of the Geophysical Survey, Geophysics Division, New Zealand Department of Scientific and Industrial Research. Dr. Hatherton was born in Yorkshire and received his education at Normanton Grammar School and the Universities of Birmingham and London. In 1950 he became the first United Kingdom recipient of a New Zealand National Research Scholarship and went to New Zealand to carry out research on the magnetic properties of New Zealand volcanic rocks, for which he was awarded a Ph.D. by the University of London. Dr. Hatherton spent several seasons in Antarctica during 1955–64, and wintered over at Scott Base in 1957 as leader of the International Geophysical Year party. For this work he was awarded an O.B.E. and a Polar Medal for 1958. In 1959–60 Dr. Hatherton spent a year at the California Institute of Technology on a Commonwealth Civil Service (Harkness) fellowship. He is editor of a treatise on *Antarctica*, recently published in the United Kingdom, New Zealand, and the United States.

Promotions at Woods Hole Oceanographic Institution

In recognition of scientific achievements of major importance over the past several years, the Woods Hole Oceanographic Institution has promoted seven members of its scientific staff to higher positions. Foremost among these was the appointment of Dr. Howard L. Sanders (zoologist) to the position of senior scientist, the highest rank of scientific appointment which is made by the Institution. Other appointments were to the position of associate scientist, and these included Dr. Carl O. Bowin (geologist), Dr. John E. Gordon (chemist), Dr. Robert R. Hessler (palaeozoologist), Dr. Richard M. Pratt (geologist), Dr. John M. Teal (biologist), and T. Ferris Webster (geophysicist). Dr. Sanders has been a member of the staff of the Woods Hole Institution since 1955 when he received his doctoral degree from Yale University. His special scientific interest lies in the area of marine benthic ecology, but he has also made extensive investigations of crustacean phylogeny. Of the other members of staff recently promoted, Dr. Bowin has made intensive studies of the density distribution of the Earth's crust and upper mantle. He has also done much to develop new techniques for the use of a digital computer on board a research vessel at sea. Dr. Gordon, on the other hand, has investigated electrolyte and non-electrolyte interactions in sea water, while Dr. Hessler has been concerned with studies on the evolution and morphology of Arthropoda and deep-sea animal communities. Dr. Pratt's special interest is in deep-sea topography. Dr. Teal has concerned himself with physiological ecology, and Dr. Webster with ocean current measurements.

Recruitment to the Civil Service

The sixth report of the Estimates Committee for the session 1964–65, dealing with recruitment to the Civil Service, is discussed on p. 311, but the report is noteworthy for the memoranda submitted in evidence. Among

these, those submitted by the Civil Service Commission on the function and organization of the Civil Service Commission and that of the Treasury on recruitment to the Civil Service may be mentioned. Recruitment to the Civil Service is dealt with in memoranda by Prof. W. J. M. Mackenzie, by Mr. B. J. Holloway, by the staff side of the Civil Service National Whitley Council, and by the Acton Society Trust. The last-mentioned is supported by a further memorandum on the attitudes of civic university students towards an administrative career in the Civil Service. This is based on a survey of third-year undergraduate students at the University of Hull, undertaken following a suggestion made in the statement of evidence made to the Committee by the Acton Society Trust. A further memorandum on recruitment to the Civil Service is by the Warden of Nuffield College, Oxford, Mr. D. N. Chester. Another on the same subject by the Business Economists Group is also appended; the Institution of Professional Civil Servants has a memorandum on recruitment to the professional and technical classes; finally, one on the recruitment of girls to the Civil Service is submitted by Miss Margaret Miles.

Anglo-Romanian Agreement on Co-operation

A FORMAL agreement on co-operation has been concluded between the Royal Society and the Academy of the Socialist Republic of Romania with the signature in Bucharest by Academician D. Dumitrescu, First Secretary of the Romanian Academy, and in London by Sir Patrick Linstead, Foreign Secretary of the Royal Society. The agreement makes provision for; (1) the annual exchange of three scientists from each side for lectures and visits to specialized scientific institutions in the other country for periods of fourteen days ; (2) the annual exchange of up to two scientific research workers from each side, for a nine-month period in each case, to study fundamental problems in some aspects of the physical and biological sciences, including their applied aspects. The agreement also calls for the exchange of periodical publications of the two sides and for the encouragement of further such exchanges of publications in specialized branches of the physical and biological sciences between the respective corresponding institutions in the United Kingdom and Romania.

Eleventh Pacific Science Congress

THE eleventh Pacific science congress of the Pacific Science Association will be held at the University of Tokyo, Japan, during August 22–September 10, 1966, under the auspices of the Science Council of Japan. The inaugural meeting will be held on August 22 and the general meeting on September 3. The first week of the congress will be devoted mainly to symposia, the second to divisional meetings and the third to scientific tours. The organizers are not limiting the number of scientists attending the congress. The branches of science covered by the Pacific Science Association include: meteorology; oceanography; geophysics; geology and soil science; biology; agriculture; forestry; animal science and conservation; fisheries; marine and freshwater sciences; nutrition; public health and medical science; social sciences; anthropology; geography; scientific information and museums. Symposia and divisional meetings will be held on specific topics in these fields. There will also be two congress symposia on (*a*) population problems in the Pacific, and (*b*) air and water pollution in the Pacific area, as well as four special symposia with exhibitions and demonstrations on vessels and instruments for oceanic and freshwater research, marine park, primate biology of the Pacific area and tsutsugamushi disease. It is hoped that various meetings of other international scientific organizations will be held in Japan concurrently with the congress.

The Royal Society, as the United Kingdom representative institution in the Pacific Science Association, will be sending an official delegation of ten members to the congress. Members attending other meetings to be held concurrently with the Pacific science congress are invited to become members of the congress, and in addition any qualified scientist may attend the congress and participate in the two weeks of meetings without presenting a paper. Representative institutions have been requested to accredit all members of the congress to the Organizing Committee, and any United Kingdom scientist planning to attend is asked to inform the Executive Secretary, the Royal Society, Burlington House, London, W.1.

High-pressure Science and Technology

THE recent international conference on "High Pressures", held at Le Creusot, France, emphasized the important common interests, particularly in engineering and experimental techniques, which link all high-pressure activities. At a special meeting, British delegates felt that better interchange of information between specialized activities would greatly accelerate progress in the United Kingdom. A committee was elected to: (1) organize meetings promoting contacts between specialists in different fields at which informal discussion would be encouraged; (2) provide a source of information on high-pressure activities in Britain, and liaison with American, Continental and other groups; (3) set up an association which all interested in high pressures could join; (4) act as an advisory body to the Government and other authorities. The committee was chosen to represent a variety of interests and will be enlarged as necessary. Members are: K. E. Bett (Imperial College of Science and Technology); C. C. Bradley (National Physical Laboratory); C. H. L. Goodman (Standard Telecommunication Laboratories, Ltd., Harlow, *Acting Chairman*); W. R. D. Manning (St. Albans, Herts). All groups in Britain working with high pressures are urged to write to the committee giving a brief outline of their interests. This will enable a provisional directory of high-pressure research to be compiled which will be circulated to all who write in. It will also form the basis for the association, and for deciding the topics for the first research meetings. Letters should be sent preferably to the acting chairman, but any committee member would be pleased to answer queries.

Scientific Periodicals from the U.S.S.R.

THE 1966 catalogues of Russian newspapers and magazines procurable on a subscription basis through national agencies for Soviet books list several hundred scientific periodicals and more than 250 sections of the *Journal of Abstracts* and the *Engineering Index*, all of which are readily obtainable by Western readers if orders are placed six weeks in advance of publication. An unwelcome feature of these 1966 lists is the rising cost of Russian journals of an academic or technical character, the price of which in most instances has advanced by 18–19 per cent since 1963. Annual charges for volumes of the *Journal of Abstracts* (*Referativnyi Zhurnal*) series have gone up by some 30–40 per cent in the same period. In numerous instances these Russian scientific periodicals, characterized a decade ago by their relative cheapness, are now appreciably more expensive than their Western counterparts. It is significant that no comparable advances are scheduled in the price of newspapers, popular magazines and propaganda literature.

Soils of the Mendip District of Somerset

A RECENT memoir of the Soil Survey of Great Britain, by D. C. Findlay, gives an account of the soils and land-use of the region between Weston-super-Mare and Bridgwater and extending inland to Shepton Mallet (*The Soils of the Mendip District of Somerset*. Sheets 279 and 280. Pp. viii + 204 + 9 plates. Harpenden: Rothamsted Experimental Station, 1965. 35*s*.). The area surveyed extends to 279 square miles and includes the Mendip

upland of limestone plateau at about 800 ft., surmounted by four Old Red Sandstone hills, and the flat belt of alluvium, constituting the Levels, along the eastern side of the Severn Estuary. Exposed rocks range from the Devonian to the Jurassic systems, while the superficial deposits accumulated since the Pleistocene period include spreads of estuarine and riverine alluvium interbedded with peat. Distance from the coast and increases in altitude account for wide variations in local climate and length of growing season. For example, the average rainfall is about 32 in. on the coast and 50 in. on the highest ground; sunshine and temperature display similar tendencies, while moisture-deficient periods may exist during six months on the coast but only during June and July on the Mendips. There is a short discussion of the vegetation changes brought about by clearances of the oak–ash woodlands of the uplands, by agricultural improvements in the vales and by peat cutting on the moors. Six chapters are devoted to an explanation of soil formation and classification, and to details of the mapping units, including profile descriptions and analytical data. The soil conditions are then discussed in relation to land-use and types of farming, and there is a useful appendix dealing with the land-use limitations of the soil series. There is a list of references, a subject index, and two maps.

Tropical Timbers

From Belgium comes a very detailed account of the timbers of 67 tree species of the Congo (*Publications de l'Institut National pour l'Etude Agronomique du Congo. Bois du Mayumbe.* Par Joseph Fouarge et Georges Gerard. Pp. 579+57 planches. Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1964). These are the principal timber species of Mayumbe, but not a few are to be found in the West African high forest and others are very closely related species. Each species is treated separately. The results of physical and mechanical tests are listed and notes are provided on the relative susceptibility of the timber to insect and fungal attacks. For many of the species a set of four micro-photographs illustrates anatomical characteristics, and these are accompanied by detailed descriptions. A final section to the volume is intended for timber merchants and others interested in the uses of timbers, but a word of warning is added directing attention to variations which may occur in the same species. Statistics of the physical and mechanical properties and the relative durability are tabulated and sections are given recommending timbers for particular purposes such as cabinet work, furniture, mouldings, carpentry, turnery, shingles, pulleys, sleepers, paving-blocks, piles, sluice-gates, marine constructional work, small boats, barrel-making, matches, pulping and other uses. This is a most useful reference work and the authors deserve praise for what they have accomplished.

Announcements

Prof. J. C. Wheatley, University of Illinois, has been awarded the fourth Simon Memorial Prize in recognition of his outstanding work on the properties of liquid helium-3 at very low temperatures. The Prize of £250 was established as a memorial to Sir Francis Simon and is awarded by the Committee of the Low Temperature Group every two or three years for outstanding contributions in the field of low-temperature physics.

The second conference on "The Countryside in 1970" will be held in London during November 10–12. Further information can be obtained from the Royal Society of Arts, John Adam Street, London, W.C.2.

A symposium on "The Chemistry and Technology of Polyvinyl Chloride" will be held at the Bradford Institute of Technology during November 5–6. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

A colloquium on "Diet and Diabetes", arranged by the Scottish Group of the Nutrition Society, will be held at the Royal Infirmary, Edinburgh, on October 30. Further information can be obtained from Dr. C. F. Mills, Rowett Research Institute, Bucksburn, Aberdeen.

A seminar on "Formulating International Science Policy", arranged by the Science of Science Foundation, will be held at the Foundation on November 15. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

A meeting on "Nuclear and Engineering Ceramics", arranged by the Basic Science Section of the British Ceramic Society, will be held at Harwell during October 25–27. Further information can be obtained from Dr. J. P. Roberts, Houldsworth School of Applied Science, the University, Leeds 2.

The Night Sky in November

All times are in Universal Time

Moon

New Moon	23d 04h
Full Moon	9d 04h

Conjunctions with the Moon

Venus	27d 06h, 0·7° N.
Mars	26d 05h, 2° N.
Jupiter	12d 08h, 3° S.
Saturn	3d 22h, 3° N.

Planets

Times of Rising (*R*) and Setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	*Dg* (10^6 miles)	Zodiacal position
Mercury	—	Unfavourable for observation					
Venus	*S*	18h 25m	18h 35m	18h 55m	−4·1	91	—
Mars	*S*	18h 20m	18h 10m	18h 05m	+1·4	63	—
Jupiter	*R*	19h 05m	18h 00m	16h 55m	−2·2	190	Sagittarius
Saturn	*S*	1h 20m	0h 25m	23h 30m	+1·1	399	Gemini→Taurus
						865	Aquarius

Dg is the distance of planet from the Earth on the 15th of the month

Occultations of Stars Brighter than Magnitude +6 at Greenwich

Star	*R*/*D*	Time	Mag.
69 Aqr	*D*	3d 18h 08·4m	+5·8
89 Psc	*D*	7d 02h 58·8m	+5·3
121 Tau	*R*	11d 20h 15·3m	+5·3
37 Gem	*R*	13d 04h 11·0m	+5·8
$\varkappa$ Gem	*R*	13d 21h 44·4m	+3·7
ν Vir	*D*	18d 03h 25·6m	+4·2
ν Vir	*R*	18d 04h 29·2m	+4·2

(*D*, disappearance; *R*, reappearance)

Meteors

Name	Active period	Date of maximum	Radiant	Remarks
Taurids	Oct 26d–Nov. 16d	1d–10d	54° R.A. +14° Dec.	Early stages favourable
Leonids	14d–17d	16d	152° R.A. +22° Dec.	Unfavourable because of moonlight

SECOND INTERNATIONAL CONFERENCE ON PROTOZOOLOGY

THE first International Conference on Protozoology was held in Prague in 1961, and the second at the Imperial College of Science and Technology in London during July 29–August 5, 1965. Approximately 570 delegates attended the Conference from 26 countries; 370 papers or films were read or shown.

In place of full proceedings, a shortened version of the papers was printed and given to delegates on arrival at the Conference, in a volume entitled *Progress in Protozoology*[1]. This service was much appreciated and certainly is preferable to reports of congresses, which are not produced sometimes until as long as seven years after the meeting has ended, while the abolition of formal proceedings allows speakers a greater freedom to speculate and report on very recent work.

The patron of the Conference was H.R.H. the Duke of Edinburgh; the honorary president was Prof. E. Fauré-Fremiet, and the Conference was organized by a national committee under the chairmanship of Prof. P. C. C. Garnham.

The annual meeting of the Society of Protozoologists was held under the presidency of John Corliss (Chicago) during the course of the Conference.

The Conference was divided into two parts: the mornings were devoted to plenary sessions based on single topics; the afternoons to special symposia and contributed papers in four simultaneous sessions. At the end of the afternoons, ciné films were shown. The plenary sessions were selected to give non-specialized workers the present views on the following subjects: morphogenesis and life-cycles, types and preservation of strains, genetics, biochemistry and physiology, cytology and ultrastructure, and locomotion.

The afternoon sessions were devoted to specialized subjects, including : marine protozoa, piroplasms, protozoa of invertebrates, metabolism and drug action, cultivation of Protozoa, ecology of free-living Protozoa, ecology and host–parasite relationships, and little-known parasitic Protozoa. The remainder of the contributed papers were arranged around their individual subjects, such as *Toxoplasma*, malaria parasites, coccidia, and ciliates. The day after the close of the Conference itself, a symposium was held on the classification of the Protozoa, when various schemes, originating from the Society of Protozoologists, from the U.S.S.R. and from Poland, were discussed. No finality was reached, but arrangements have been made for the continuation of this discussion by the International Commission on Protozoology. This body was formally instituted at the Conference, following a proposal by Prof. Pierre de Puytorac (Clermont-Ferrand). It has the responsibility for organizing future International Conferences, to work for the establishment of relations with other international biological organizations, such as the International Union of Biological Sciences and Unesco, and particularly to appoint various committees to consider such subjects as the preservation of types, the maintenance of culture collections, the revision of systems of classification of the Protozoa, and the participation of protozoology in the International Biological Programme. The Conference itself passed a resolution requesting that the World Health Organization should be invited to convene a meeting for discussing how international co-operation could assist in the preservation of strains and types of Protozoa. The Conference also resolved that an abstracting service for protozoology is highly desirable, and the feasibility of the provision of this service is to be investigated. The final resolution of the Conference was that the third International Conference on Protozoology should be held in Leningrad in 1969.

The importance of the Protozoa in the whole field of genetics was emphasized by Sonneborn (Indiana), who pointed out that while work on viruses, bacteria, yeasts, etc., appeared to have overshadowed that on Protozoa, it was nevertheless the protozoologist who, in the past, had pioneered a number of important routes for use in general genetics. The Protozoa are likely to become increasingly important in this field, by virtue of their special suitability for investigating certain types of genetical activity, including the special role they have in cytoplasmic genetics. This aspect was further developed by Preer (Philadelphia) in a discussion on *Kappa* and its relatives; he showed that the mate-killing *mu* particle is maintained by the gene *M*, by producing "'metagons'—particles which prevent destruction of *mu*. Metagons in extracts prevent loss of *mu* in metagon-depleted paramecia. The latter are produced by treating mate-killers with ribonuclease or replacing *M* genes by *m* genes using suitable crosses and autogamy. Metagons are present in the RNA extracted from ribosomes of paramecia bearing the *M* gene. Metagons hybridize well with DNA from paramecia containing *M* genes and poorly with DNA from paramecia containing *m* genes. Consequently metagons are thought to be messenger RNA. Metagons from paramecia, when introduced into certain ciliates such as *Didinium*, become self-reproducing, like RNA viruses". Recent Russian work, for example, by Judin (Leningrad), involving nuclear transplantation and amoebae, appears to minimize the control of genetic effect by the cytoplasm; he concluded that the inheritance of all the characters studied is conditioned exclusively by the nucleus. Grell (Tübingen) explained the new ideas on sexuality in foraminiferans, in particular autogamy or the mating of gamonts, which could be differentiated into two morphologically similar types, reacting differently in the course of their lives.

There were numerous contributions from the French School (headed by Fauré-Fremiet, de Puytorac, Hovasse, Savoie, Mme Noirot-Timothée and others) on the ultrastructure of the ciliates, and the structures were not merely described, but also considered from the point of view of their nature, morphogenesis, and mode of action.

The paper by Satir (Chicago) presented evidence for a sliding filament mechanism in the peripheral fibres of flagella and cilia. The 'doublets' consist of a longer and shorter filament; the shorter filaments are apparently different in the two cases and they are always distal to the cell of origin compared to the longer filaments. This agrees with expectations for sliding-filament models of filament function where the filaments at the bottom of the cross-section would move out past those at the top to accommodate curvature.

Progress has also been made in other aspects in the study of flagella and cilia. The great versatility of these organelles, especially in relation to the presence and arrangement of mastigonemes, was stressed by Jahn (Los Angeles). The mathematical analysis of the hydrodynamic principles involved in both flagella and cilia has advanced considerably, as illustrated by the papers of Rikmenspoel (New York), Holwill (Dorking) and Machin (Cambridge). Further, the co-ordination of ciliary action was shown by a number of speakers to be mediated mechanically and not, as has often been assumed, by the transmission of neuromotor impulses by infra-ciliary fibres. The Polish workers in particular (Dryl, Grebecki and others) reported extensive experimental work correlating various forms of ciliary activity with surface potential, thereby providing a physiological interpretation of ciliate movement and reaction (for example, the induction of anodal galvano-

taxis in *Paramecium caudatum* by membrane calcium). Mendelson and Warmouth (Boston) made an attempt to demonstrate learning in *Paramecium caudatum*, in which repeated mechanical stimuli, paired with light or absence of light, were presented to individual animals in a designated region of a capillary test chamber. The animals avoided the area in which the mechanical stimuli were given only under conditions of low-intensity illumination. Although avoidance persisted for a period of several minutes following cessation of mechanical stimuli, this response cannot be interpreted as learning since it was not dependent on pairing of any other stimulus with the mechanical stimulus. Further, during stimulation of one animal, a second animal also present in the chamber, but not receiving the stimuli, developed the avoidance response to the area in which the first animal was stimulated. Since it is unlikely that mechanical stimulation produces changes directly in the medium, it is suggested that a material is elaborated by paramecia in response to noxious stimulation. A simpler aspect of locomotion was beautifully demonstrated by Freyvogel (Basle) in a speeded up ciné-film to show the movements of malarial ookinetes. In recent years their inherent motility had been denied. The film clearly demonstrated that two types of movement are to be distinguished: in the first the ookinete is attached to the substrate with one pole as a sucker; with the free part of the body it carries out circling movements, but it remains on the spot. By the second a more or less even locomotion is made possible; this is brought about by circular contraction waves which originate at the anterior pole and which migrate on the surface of the body, well over its central portion. With reference to the substrate these contraction waves remain on the spot and the protoplasm with its inclusions is driven forward through them. In this way, on one occasion, an ookinete was shown to cover about 100μ within 40 min.

Few papers dealt with amoeboid movement, doubtless because this has been the subject of a recent symposium elsewhere, but the demonstration by Seravin (Leningrad) that amoeboid locomotion is produced not by a single but by many physiological mechanisms strikes a new note.

While the importance of the organelles, as revealed by electron microscopy, is fully recognized, Cheissin (Leningrad) pointed out that their presence or absence should not be regarded as an indication of the taxonomic position of the organism concerned; such organelles have a common function throughout the animal and plant kingdoms, and their existence is an indication of function rather than of taxonomy. On the other hand, as Jahn (Los Angeles) states, locomotion itself has always been regarded as the most important and generally accepted single taxonomic criterion of non-fossil Protozoa from subphyla to species, and this function of the organism is dependent on such organelles. Polyansky (Leningrad) extended these ideas in an interesting discussion on evolution in the Metazoa and Protozoa; in the former, there is a tendency to a decrease in the number of organs, in the latter an increase (polymerization). This is accomplished either by increasing their actual number (for example, the nuclei of opalinids), their duplication as in *Giardia* or their differentiation into two types as in the macro- and micro-nuclei of ciliates. A further extension of the process is by polyploidization with the emergence of new evolutionary forms.

The foregoing topics largely concerned the free-living Protozoa, although many of the conclusions can also be applied to the parasitic forms. The latter represent specialized subjects, progress on which has advanced since the preceding conference on predictable lines.

The subject of piroplasms was selected as representing an area where important gaps in knowledge exist, in the hope of stimulating fresh work, but unfortunately little major progress in relation to the life-history of these organisms or even on their taxonomic position has yet been effected.

It was obvious that a great deal of work is being done on the biochemistry of the Trypanosomatidae, and this is commencing to throw light on their phylogeny and classification. Guttman (New York) explained the different nutritional requirements in special media of *Leishmania*, *Leptomonas* and *Crithidia*.

Artificial immunization of animals against the malaria parasite (Margaret Weiss, Michigan) and the trypanosomes (Soltys, Cambridge) has at last been accomplished. In the former case, mice were actively immunized by the injection of a strain of *Plasmodium berghei* which had become non-invasive for mice. This strain had lost its infectivity for mice, though not for rats, following a number of sojourns in tissue culture in a medium containing hamster serum. An initial immunizing dose containing few parasites was followed 2 weeks later by a booster injection containing 1–2 million parasites of the same strain. The mice were afterwards challenged by the parent strain which kills control mice within 3 weeks. Immunity was slow to develop, and during an initial period extending for about 4 weeks after the booster injection the mice were particularly susceptible, and succumbed even faster than the controls to challenge infection with the virulent strain. But after this sensitive period good immunity developed which was solid in many cases; in others single parasites were seen, and in a few cases peak parasitaemias of 1–2 per cent red blood cells parasitized were observed. This degree of immunity persisted for about 4 months. Soltys showed that *Trypanosoma brucei*, inactivated by treatment with β-propiolactone, could be used as a living vaccine to protect mice against the homologous, virulent strain.

The recent discovery by Mme Landau (Paris) of a new species of rodent malaria parasite is likely greatly to facilitate malaria research, because *Plasmodium chabaudi* represents a model where an infection in a small laboratory mammal can be used with much greater ease and success than the well-known *P. berghei* in mice or *P. cynomolgi* or *P. knowlesi* in monkeys. The latter systems present many technical difficulties; on the contrary, the new species is easily transmitted by mosquitoes and enormous numbers of exoerythrocytic forms are produced. This work stems from the highly successful recent experiments of Yoeli[2] (New York) on the transmission of *P. berghei* and the discovery of its exoerythrocytic cycle in the liver of mice and hamsters.

Further knowledge of the ultrastructure of the Sporozoa was advanced by Scholtyseck's (Bonn) work on sporozoites of *Eimeria perferans*, Cheissin's (Leningrad) on *E. bovis*, Sheffield and Hammond's (Utah) also on *E. bovis*, Vivier's (Lille) on gregarines and coccidia, and Bardele's (Tübingen) on *Eucoccidium dinophili*.

A special topic of the first Conference was *Toxoplasma*; little new information has emerged in the past four years relating to the life-history of this organism until very recently when Hutchinson (Glasgow)[3] described the apparent transmission of *Toxoplasma* through the egg of *Toxocara catti*. He did not give a paper on this subject, but discussed the implications in the session dealing with the organism.

A newly discovered disease of man, due to the free-living amoeba, *Hartmanella castellanii*, was described by Culbertson (Indiana), who gave details of his experimental work with this organism in rabbits and mice, and showed slides of the organism as found in the tissues from 7 fatal human cases.

The role of *Pneumocystis carinii* as a pathogen of man becomes more formally established following papers by Kučera (Prague), Frenkel (Kansas) and Yaeger (New Orleans).

P. C. C. GARNHAM

[1] *Intern. Congr. Ser.*, No. 91 (Excerpta Medica Foundation, Herengracht 119–123, Amsterdam, C).

[2] Yoeli, M., *Trans. Roy. Soc. Trop. Med. Hyg.*, **59**, 255 (1965).

[3] Hutchinson, W. M., *Nature*, **206**, 961 (1965).

TYPOGRAPHY OF BACTERIA

AT an informal meeting of editors of journals in which bacteriological papers appear, and some other interested persons, it was agreed that a greater uniformity in typography was desirable; the following proposals, which arose from the meeting and from subsequent discussion, may be of wider interest.

It must be made clear that these proposals are concerned solely with typography and not with the more controversial subjects of classification or nomenclature.

(1) Nomenclature. The International Code should be followed. (*International Code of Nomenclature of Bacteria and Viruses: Bacteriological Code:* Ames, Iowa, Iowa State University Press, 1959.)

(2) Major ranks, such as the names of orders, tribes or families, are written in roman with an initial capital; for example, Enterobacteriaceae.

(3) Binomials consisting of genus and species together are italicized, only the genus having a capital letter; for example, *Staphylococcus aureus*. [Note: A binomial which is considered invalid may be enclosed in quotes; for example, '*Bacillus typhosus*'.]

(4) Sub-species (varieties) are italicized, without a capital, and preceded by 'subsp.' (or 'var.') in roman; for example, *Corynebacterium diphtheriae* subsp. *mitis*.

(5) Strains are printed in roman and are capitalized only when derived from proper names; they are preceded by 'strain' in roman or may be regarded as part of a trivial name (see 9); for example, *Staphylococcus aureus* strain Oxford, the Oxford staphylococcus.

(6) Groups and types are printed in roman and designated by capital letters and arabic figures respectively; for example, *Streptococcus pyogenes* group A type 1.

(7) Generic names are italicized either when used alone to mean the whole genus as a genus or followed by 'sp.' or 'spp.' to indicate a culture which has been identified only as far as the genus. Otherwise when used either as nouns or adjectives they are regarded as trivials and printed in roman; for example, the genus *Brucella* . . ., culture yielded *Bacillus* sp., some escherichias may have salmonella antigens.

(8) Specific epithets are occasionally used alone, and are usually regarded as trivials and printed in roman; for example, the strains were of the abortus type.

(9) Trivial names should be in roman type and capitalized only when derived from proper names. (Not when names of diseases are used.) Generic names may be used trivially; for example, meningococcus, Koch–Weeks bacillus, haemophilus, spirochaete, typhoid bacillus.

(10) Anglicized plurals may be used for trivial names to avoid classically derived forms which may appear pedantic or clumsy; for example, salmonellas, mycoplasms, actinomycetes, pseudomonads, leptospires.

(11) Hyphens should be used sparingly and never in italicized binomial (or trinomial) forms. A single word must be used to avoid confusion; for example, *Salmonella choleraesuis*, *Bacillus pseudotuberculosisrodentium*; but note: *Salmonella paratyphi B*.

(12) Abbreviations should be used with caution. Generic names may be abbreviated to a single capital letter except: in titles; in summaries; when first mentioned in the text and when any possibility of confusion could exist; for example, *S. aureus* (see Cowan, S. T., *Science*, 120, 1103; 1954); specific epithets should never be abbreviated, except possibly in tables.

(Note: Special *ad hoc* abbreviations may sometimes be acceptable in text-books or abstract journals, so long as they are clearly explained in the text.)

(13) Salmonellas present a special problem as their nomenclature is still in dispute. It is not the purpose of this communication to discuss the merits of the various proposals but to suggest appropriate typography. The following examples illustrate some of the ways in which the same culture may be described. (*a*) A separate species, and so written as a binomial in italics with the specific epithet in latinized form and in lower case; for example, *Salmonella londonensis* (this example is after Haupt, cited in *Bergey's Manual*, sixth ed. The latinized form of the word 'London' should, in fact, be 'londiniensis'). (*b*) As above but with the specific epithet unlatinized; for example, *Salmonella london*. (*c*) A variety of a large inclusive species and so written with the epithet in lower case italics; for example, *Salmonella kauffmanii* var. *londonensis*. (*d*) A serotype of a large inclusive species and written in roman with an initial capital if derived from a proper name; for example, Salmonella kauffmannii [serotype] London. (*e*) A trivially named serotype written in roman throughout with an initial capital if indicated; for example, Salmonella London.

(14) Notes: (i) Specific epithets should be a single word in lower case, but serotypes, being trivials, may have more than one word and capitals where required. See pars. 9 and 11; for example, *Salmonella saintpaul*, c.f. Salmonella Saint Paul. (ii) A hybrid or bastard composed of an italicized generic name and a roman serotype cannot be defended and should not be used; for example, *Salmonella* London.

(15) The nomenclature of salmonellas is discussed more fully by Cowan, S. T. (*Bull. Hyg.*, 32, 101; 1957). Here, we would merely suggest that wherever possible a single usage should be adopted for use in a single communication.

A Note on the Typography of Other Micro-organisms

(16) Fungi and protozoa. Nomenclature is governed by the international botanical and zoological codes. In general, our suggestions for bacterial typography could apply.

(17) Viruses. The nomenclature of viruses is at present being considered by the Provisional Virus Nomenclature Committee of the International Association of Microbiological Societies. Until their findings have been accepted and approved we consider that names of viruses should be regarded as trivials. The following tentative suggestions for their typography may be helpful. (*a*) Viruses named after a disease should be written as separate words in lower case roman, except where the name of the disease is derived from a proper name; for example, measles virus, poliomyelitis virus, varicella-zoster virus, herpes simplex virus, Newcastle disease virus. (*b*) Viruses named after a place or person should be written as separate words with an initial capital; for example, Coxsackie virus. (*c*) Classical or euphonious synthetic prefixes should be combined with the suffix 'virus' and written as a single word in lower case; for example, adenovirus, cytomegalovirus, echovirus, picornavirus, poliovirus. (*d*) Unpronounceable synthetic prefixes should be written as a separate 'word' entirely in capitals; for example, LCM virus.

Among those who make these recommendations are: Sir Christopher Andrewes, formerly deputy director, National Institute for Medical Research; Dr. H. J. O'D. Burke-Gaffney, editor, *Bulletin of Hygiene*; Dr. S. T. Cowan, director, Central Public Health Laboratory, formerly curator, National Collection of Type Cultures; Dr. R. M. Fry, editor, *Journal of Hygiene*; Dr. J. C. Kelsey, editor, *Monthly Bulletin of the Ministry of Health and Public Health Laboratory Service: Section II*; Prof. C. L. Oakley, editor, *Journal of Pathology and Bacteriology*; Mr. A. F. B. Standfast, editor, *Journal for General Microbiology*; Mr. G. Sykes, editor, *Journal of Applied Bacteriology*.

ANGLO-AFRICAN JOINT RESEARCH SCHEMES AND RECRUITMENT

A CONFERENCE was held under the auspices of the Centre of African Studies, University of Edinburgh, in association with the Ministry of Overseas Development, on "Joint Research Schemes and Recruitment", at the University of Edinburgh during September 13–17.

Ninety delegates from universities, training colleges and colleges of advanced technology in Scotland, the North of England and Northern Ireland and Africa attended the conference. Representatives came from thirteen British institutions of higher education and fourteen African, together with representatives of the British Ministry of Overseas Development, the Commonwealth Association of Architects, the Architectural Association, the British Council and the Inter-University Council for Higher Education Overseas.

Although this conference was called originally to discuss joint research schemes between institutions of higher education in Scotland, the North of England and Northern Ireland and African universities and also recruitment in this northern region of Great Britain for African universities, it soon extended its terms of reference to include almost the whole field of relations between British and African higher educational institutions. This tendency undoubtedly grew from the fact that both the British and African universities find themselves in an exciting period of parallel development in a rapidly changing world.

In the past it has been common to consider that the relations between British and African universities were heavily weighted on the side of British initiatives. It soon emerged from the conference, particularly as a result of the contributions of the African delegates, that this was a two-way traffic and that the British universities would also benefit from an extension of their relations at all levels with African universities.

The conference, in its conclusions, stressed the necessity of improving, in all their ramifications, the channels of communication between British and African universities. Not only was it necessary to improve the existing channels of communication between the Ministry of Overseas Development, the Inter-University Council for Higher Education Overseas, and British and African higher educational institutions and to improve the information services on African educational possibilities in Great Britain of British professional organizations, but it was also important to set up regional committees of British universities to improve their own communications with African universities. In particular, there should be a committee of the Scottish, North of England and Northern Ireland universities, training colleges and colleges of advanced technology for this purpose.

A major decision of the conference—one in which it struck out in a new direction in British–African higher educational relationships—was that throughout the British Isles a number of committees based on the major academic disciplines and organized on trans-university lines should be set up to initiate new joint research schemes and activities between British and African academic institutions and to review existing joint activities periodically.

The conference urged that a directory of research in progress in African universities should be produced as soon as possible. Furthermore, it urged that a directory of specific post-graduate opportunities in British universities (their individual research interests and achievements; the facilities which they offered for research; the size of their post-graduate departments; and their reputations in their respective fields) aimed at the particular needs of African post-graduate students should be compiled as soon as possible. This, it was suggested, should be revised frequently, in order to present the potential African postgraduate student with as up-to-date a picture as possible of postgraduate prospects in a rapidly changing scene.

The conference stressed strongly the importance of both official and unofficial funds being made available, not only in improving the conditions of secondment of British academic workers in African universities but also for improving the position of the permanent staff, many of whom were from Great Britain, in African institutions of higher education. The financial implications of secondment from British to African universities should give greater attention to ensuring that seconded personnel do not lose financially, as is so often the case, during their period of secondment to an African university. Careful safeguards to this effect should be written into their agreement with an African university. Greater efforts should be made at the British end to supply African universities with technicians and 'middle level' administrators and secretaries.

Secondment of senior personnel (such as deans of faculties and heads of departments) from British to African universities, it was considered, should be for periods sufficiently long to ensure that programmes which such individuals initiated in African universities were properly launched.

The conference directed special attention to the problems of African post-graduate research students and stressed that greater attention should be given to placing them in those British universities in which their interests and talents could be most effectively developed. Departments in British universities should be discouraged from assuming responsibility for African postgraduate students for whom they could not provide viable research topics, adequate supervision and the necessary materials for research.

In its detailed consideration of joint research schemes between British and African higher educational institutions—many of which were developed in detail in a series of inter-disciplinary discussion groups—the conference stressed that British universities should endeavour to participate in existing joint research schemes with African universities and in schemes which have been initiated in African universities themselves. While recognizing, however, that such approaches are less wasteful in time, money and manpower than joint research schemes launched independently in Great Britain, it was considered important not to discourage independent schemes in those fields where British institutions of higher education were in a position to give a lead to African universities.

The conference urged that experiments should be made by the universities of Scotland, the North of England and Northern Ireland in setting up international halls of residence and British–African halls of residence in order to go some way towards ameliorating the growing crisis in student accommodation in British universities.

The extent of the conference's detailed deliberations will be reported in the *Proceedings* which it is hoped to publish shortly. In anticipation of these, it should be indicated that those deliberations ranged into unexpectedly detailed fields. For example, there was a lively discussion on the necessity of continuing the external examiner system in African universities. Again, it was suggested by a considerable number of individuals at the conference that there were certain fields such as African history in which external examiners could be used from African universities in British universities.

Finally, the conference expressed a strong wish that the many recommendations for improving relations between British and African universities which had been made in its various sessions should be translated into action as

soon as possible at all levels, both institutionally and individually. The conference saw in the suggestion indicated here that academic disciplinary committees should be established for this purpose, one of the most hopeful signs for the future. It was considered that in their meetings in the future such academic disciplinary committees should consider the possibility of holding further conferences of the kind held in Edinburgh.

MODERN TECHNIQUE IN RESTORATION OF ROMAN MOSAICS

THERE is an inborn fascination to many of us, besides archaeologists and historians, in the discovery and unearthing of relics of life as it existed centuries ago. None of these is perhaps more generally exciting and popular than the Roman mosaic pavements, those skilfully constructed floorings in regular cubes of stone of many colours, beautifully contrived in patterns and pictures, frequently found in western Europe, far less common in Britain. Proof of life as it existed in Yorkshire during A.D. 100–400, as evidenced by the discovery of two excellent examples of Roman mosaic handiwork, is furnished by the Rudstone pavement, a few miles inland from Bridlington (1933), and by one at Brantingham, near the north bank of the Humber, a few miles west of Hull, unearthed in 1962. Some faithfully illustrated records of these two mosaics and particularly the manner of their preservation are contained in a recent publication entitled *Araldite in the Restoration of Roman Mosaics**.

At Rudstone three mosaics were originally uncovered and housed on site for many years. However, they began to deteriorate and were ultimately removed to Hull Museums for permanent preservation, this work being undertaken by the Museum staff and successfully completed in 1962. "One mosaic, 12¾ ft. × 10¾ ft., has a figure of Venus as its centrepiece; another, 11 ft. by 11 ft., shows a geometric pattern, while the third, depicting marine life, measures 10 ft. × 5 ft. These panels are now beautifully displayed in the museum." Their removal from site was, however, a matter of great concern and cost. The geometric panel, for instance, with its steel reinforcement, weighed 4½ tons; its display on a wall entailed the use of temporary scaffolding for its erection, a costly and laborious job. The lessons had been learnt with these Rudstone mosaics the hard way; when it came to the question of lifting the newly discovered Brantingham pavement, different methods of preservation were resorted to, involving the use of an 'Araldite' (CIBA) epoxy resin formulation and a novel technique.

The Brantingham pavement apparently formed the floor of the largest room in what is thought to have been a distinguished Romano-British villa. It originally measured 36 ft. × 26 ft. It is the largest example of Roman mosaic work so far discovered in Yorkshire. Unfortunately part of this mosaic had suffered damage by collapse into flues of an underlying hypocaust, but a number of large panels were well preserved, removed, and are now in course of preparation for exhibition in Hull Museums. Some smaller sections of the mosaic were taken away flat, but other larger portions were transported by what is known as the 'rolling-up' technique. Briefly, the procedure is as follows: to ensure absence of moisture, a metal scaffold frame is erected over the pavement and on this a plastic sheet is stretched; butane gas heaters are then directed on to the pavement for some three days or so; at the end of this drying, two coats of a polyvinylchloride solution are spread over the mosaic, a bandage material laid thereon, and a top sealing coat of polyvinylchloride. The next operation—a difficult one—is the actual rolling up of the pavement; according to this account: "This was done on a cardboard roller, 2 ft. in diameter, strengthened internally and filled with a suitable axle. The tesserae (the individual stone cubes forming the pavement) were cut away from the mortar with long iron blades and several workers helped to turn the roller and ensure that the pavement was evenly wound. As they went on to the roller, the layers were interleaved with two sheets of corrugated paper and a strong dust sheet which was pulled tightly over the tesserae to keep them in place". Thus the sections of the pavement are transported to the museum.

The unrolling stage is the next operation and it is here that the 'Araldite' resin plays its part. The major sections of the pavement are unrolled face down on a sloping surface; the edges are boxed in with timber to make a container for the backing; any loose mortar is cleaned off; then an 'Araldite' coat is applied together with chopped glass fibre. Finally, this is backed up with 2 in. of vermiculite and iron reinforcing bars bonded with an 'Araldite' resin formulation. The whole process results in a light but rigid structure. Compared with the Rudstone mosaic panels, it is estimated that this technique results in the weight of the Brantingham panels being less than a quarter per unit area.

The great advantages of these cured 'Araldite' epoxy resins for this sort of work are their stability, resistance to chemical attack and moisture. Furthermore, they possess strong adherent properties to a host of different materials, such as stone, glass fibre and vermiculite. It is indeed no boastful claim that ". . . these beautiful pavements are now permanently preserved for the benefit of posterity . . ."

* Ciba (A.R.L.), Ltd., *Technical 'Notes'* (July, 1965): *Araldite in the Restoration of Roman Mosaics*. Pp. 12. (Duxford: Ciba (A.R.L.), Ltd., 1965.)

A PROPOSAL ON NATURE'S TIME-SCALE

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IT has become increasingly obvious through the various cosmological and relativity theories that we live in a non-linear universe. As has been aptly pointed out[1], there is a tendency to make simplifying assumptions about all observable phenomena in order to explain them in a linear manner. In a way this has lulled us into the belief that most processes may be considered to exist in some theoretically idealized state. Hence, many observations of non-linear processes are compromised by considering ". . . equations to be linear for a sufficiently small displacement . . ." or, ". . . in a certain range the curve can be approximated by straight lines as shown". However, these idealized assumptions (that is, homogeneity, frictionlessness, weightlessness, temperature of absolute zero, perfect gas, perfect vacuum, etc.) are never observed in reality, and in all likelihood never occur in Nature. Even though such assumptions may aid greatly in understanding the mechanisms involved, they do not lead to a complete description of the process. As stated by D. W. Thompson[2] in a discussion concerning the regeneration of tadpole

tails: ". . . it is obvious that the value which we have found for the latter portion of the curve (however closely it be conformed to) is only an empirical value; it has only a temporary usefulness, and must in time give place to a formula which shall represent the entire phenomenon, from start to finish".

This communication proposes a non-linear function of time which appears to describe the time-course relationships of a variety of processes.

Several proposals have been made recently on re-evaluating some of the basic units of measurement. Baldwin and Tonks[3] propose the adoption of a logarithmic pressure scale which would encompass both extreme vacuums and high pressures with equal facility. The authors emphasize the advantages of converting to a logarithmic scale and refer to the established precedence for such a scale in the *p*H system. This scale would permit the recording of vacuums approaching that of an absolute vacuum, a limit (it should be emphasized) which has never been attained experimentally. Likewise, a proposal for the alteration of the temperature scale has been advanced by Georgian[4] in which he maintains that new units are necessary to describe the proper dimensions for temperature, that is, specific energy. When the proper conversion is made, the gas constant, R, becomes unity and the Boltzmann constant, k, is recognized as the reciprocal of the Avagadro number. In addition to the resulting simplification it may be appropriate to consider the temperature scale as logarithmic also. As in the case of an absolute vacuum, a temperature of absolute zero is a limit which may be closely approached but never attained. The adoption of logarithmic scales would provide a continuous connecting link between the infinitesimally small and the infinitely large.

Proposals which consider the nature of macrocosmic time as exponential or logarithmic functions have been advocated by Milne and by others[5–7]. A logarithmic nature to psychological perceptions of time has also been noted[8,9]. If time is truly non-linear then this non-linear function should be equally applicable to processes describing microcosmic events. In whatever process the time parameter is involved there should exist some descriptive function which incorporates a non-linear function of time.

An investigation of the kinetics of germination of bacterial spores has led to the derivation of an empirical equation which accurately describes the sigmoidal time-course curves obtained[10–12]. The equation, $Y = \exp(-kt^{-c})$, may be converted into a linear function by plotting $\log \log 1/Y$ versus $\log t$, where Y is the fraction of the process completed in time t, k is a function of the intercept at $\log t = 0$, and $-c$ is the slope.

The equation also successfully describes the sigmoidal time-course curves obtained from a variety of different processes. Further applications suggest that time-course curves which appear to be hyperbolic rather than sigmoidal are also described by the equation. Representative types of time-course curves from a variety of processes are shown in Fig. 1. The time-scale is expressed in arbitrary units depending on the nature of the specific process being measured. Curve A is representative of processes which appear to reach completion very rapidly, such as fast chemical reactions and, if inverted, even survival curves. Curves B and D represent processes describing rectangular hyperbolas passing through the origin, such as observed with many enzymatic and chemical reactions. Curves C and E represent processes which describe sigmoidal time-course curves such as growth, certain chemical reactions, spore germination, enzyme induction, and a whole host of other naturally occurring phenomena. Curve F represents the type of process which appears to be linear with time, such as one which completes only a very small fraction of the total process during the period of observation. The curves shown in Fig. 1 were all generated by use of the equation by assigning to the constants, c and k, the values given in Table 1.

Table 1. THE VALUES OF THE CONSTANTS USED IN THE EQUATION $Y = \exp(-kt^{-c})$ TO GENERATE THE CURVES IN FIG. 1

Process	c	k
A	3·725	0·0002286
B	0·6502	0·3558
C	1·536	3·224
D	0·4307	1·709
E	1·096	6·909
F	0·3010	4·606

Because of the variety of processes which have been examined and found to conform to the equation, the following premise is suggested: the time-course curves of all natural processes are sigmoidal whether or not an induction period or inflexion point can be detected during a particular observation. Examination in the classical sense of curves A, B and D in Fig. 1 suggests that a tangent to the curve at the origin might be constructed and that the slope of such a line would give the maximum rate of the process. These suppositions have been extensively utilized in the formulation and development of classical kinetic theory. The same argument holds for curve F in which it appears that a straight line passing through the origin may be drawn through the points. If the foregoing premise is true, however, then all the curves actually must possess an inflexion point at a time other than zero although it may approach zero to any degree desired. Since each of the curves depicted was generated by the equation $Y = \exp(-kt^{-c})$, the inflexion points may be estimated mathematically[12]. Inspection of curves A, B and C suggests that they tend toward a limit of 1·0. However, this limiting value is not as apparent with the remaining curves. Curve F may appear either to increase indefinitely (if considered a straight line) or to approach a limit significantly less than a value of 1·0 (if curvature is acknowledged); however, the equation used to generate each curve is based on a limiting value of 1·0 as time approaches infinity. Curves D and E also appear to approach limits somewhat less than the maximum of 1·0 even when the usual methods of extrapolation to infinity are employed. It can be estimated by means of the foregoing equation that process F would require $2{\cdot}82 \times 10^5$ units of time to reach 90 per cent of completion, process D would require 750 units of time, and process E, 64 units of time. Thus, the equilibrium value of a process determined by application of the foregoing equation may be appreciably greater than the value estimated by extrapolation procedures at present available.

Investigation of the possible physical significance and properties of the equation has led to the formulation of the

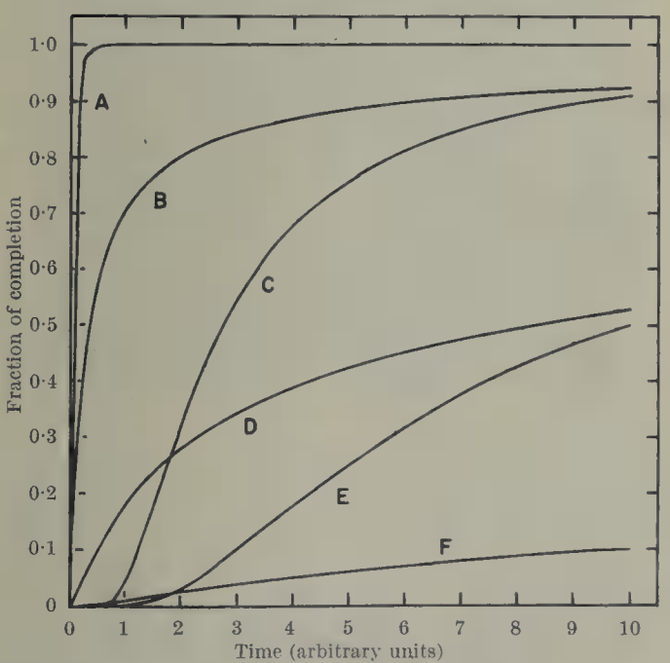

Fig. 1. Time-course curves typical of various types of processes generated by the equation $Y = \exp(-kt^{-c})$. The corresponding values for the constants are given in Table 1

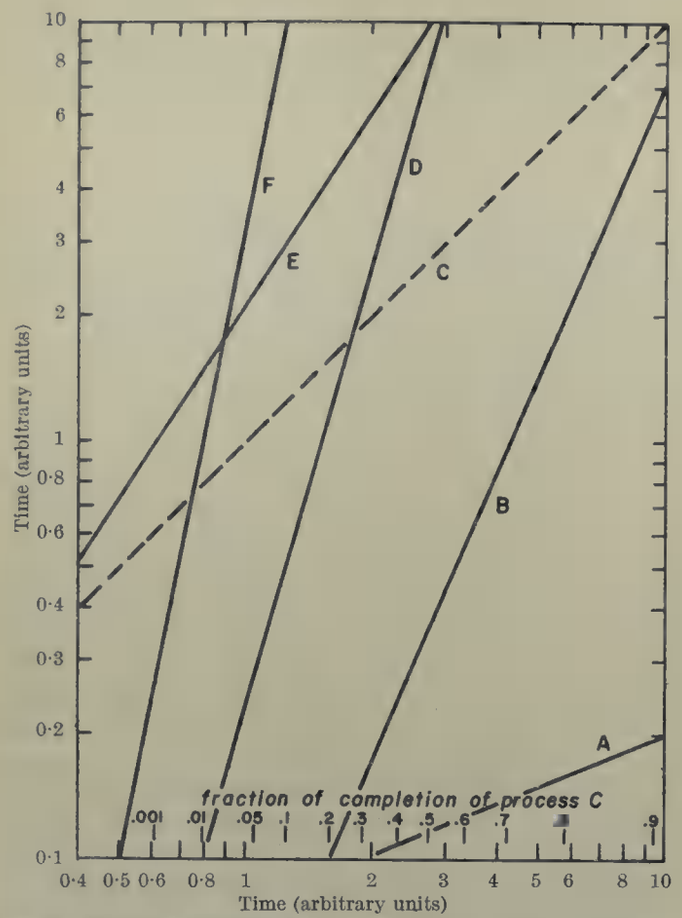

Fig. 2. Exponential relationships of the times required for processes *A*, *B*, *D*, *E*, and *F* to attain various fractions of completion versus the time required for the reference process (*C*) to achieve the same fraction of completion

following hypothesis: all naturally occurring phenomena proceed according to exponential functions of time and each process in Nature conforms to its own unique time scale. The refutability of the hypothesis may be examined by testing a corollary to the hypothesis; namely, that a process need not necessarily be timed by a clock, but may be 'clocked' by reference to any other reaction in Nature. That is, the time required for any specific process in Nature to attain a certain degree of completion (or fraction of its limiting value) is exponentially proportional to the time required for any other natural process to attain the same fraction of completion. This is illustrated in Fig. 2. The times required for the process represented by curve *C* (the reference 'clock' in this case) to attain various fractions of completion (superimposed scale) are plotted along the abscissa while the times required for each of the other processes to reach the respective fractions of completion are plotted along the ordinate. If a complete and accurate record of the reference process is known, and if the extent of completion of the second process is known at two different times then the course of that process may be predicted for any time. The dashed line represents a guideline of the reference curve *C* plotted on both the ordinate and abscissa.

The final proof of the hypothesis lies in testing the foregoing corollary with accurate data from a wide variety of different processes. In order to calculate the fraction of completion at any time and to apply the experimental data, it is imperative that the exact zero time be known as well as the true limiting (or equilibrium) value of the process as time approaches infinity.

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SUBCUTANEOUS SARCOMATA IN HAMSTERS INDUCED BY ROUS SARCOMA VIRUS (BRYAN)

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WHEN the investigation reported here was begun (June, 1964) the Bryan strain of the Rous sarcoma virus (RSV) had been found to be oncogenic for fowl only, while several strains of RSV, including the Carr–Zilber and Schmidt–Ruppin, produced tumours in a variety of mammalian species[1–3]. The experiments of Stewart and Landon (reported by S. E. Stewart at the Stern symposium, "Perspectives in Virology", February 1964) suggested that RSV (Bryan) could be 'activated' to be oncogenic for mammals by suitable treatment with a hamster embryo

Table 1. Incidence of Tumours following Inoculation of Rous Sarcoma Virus in New-born Hamsters

Group	Inoculum	Pock-forming units	Animals	Incidence of tumours (days)						Died	+	−	% +
				0–14	15–20	21–30	31–60	61–90	91–200¶				
A	RSV (Bryan)*	2×10^4	21	0	0	1	0	0	1	1	2	18	10
B	RSV (Bryan)† reconstituted	1×10^5	122	0	1	12	4	2	7	25	26	71	27
C	RSV (Bryan) plus hamster embryo extract‡	1×10^5	37	0	8	2	1	0	6	2	17	18	49
D	RSV (Schmidt-Ruppin)§	$2{\cdot}5 \times 10^2$	24	0	2	2	0	0	4	2	8	14	36
E	RSV (Schmidt-Ruppin)§	1×10^4	71	0	1	3	12	8	18	10	42	19	69
F	Hamster embryo extract		40							11	0	29	0
G	Eagle's basal medium		71							1	0	70	0
H	No injection		37							0	0	37	0

* Combined data from several experiments using *CT* 951, *CT* 955 and *CT* 957. Original sample (in 0·05 M sodium citrate) diluted with Eagle's basal medium plus 5 per cent calf serum.
† Bryan stocks, pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum.
‡ See text for preparation of sample.
§ Schmidt–Ruppin strain, Lots 11 and 29, University Laboratory, Highland Park, New Jersey. Samples diluted with Eagle's basal medium plus 5 per cent calf serum.
¶ The experiments were terminated at 200 days.

extract[4]. These findings led to an investigation in our laboratory in which RSV (Bryan) was inoculated into new-born hamsters under several experimental conditions including treatment with hamster embryo extract. We have found that RSV (Bryan) can yield sarcomata in hamsters following inoculation (10^4–10^5 pock-forming units in 0·1 ml.) in new-born hamsters in the absence of any extract from rapidly growing tissue. Since these experiments were started, Rabotti *et al.* have reported the formation of gliomas and choroid plexus papillomata induced by intracerebral inoculation of RSV (Bryan) in new-born hamsters[5].

RSV (Bryan, lots *CT* 951, *CT* 955 and *CT* 957) was provided by Dr. W. Ray Bryan, National Cancer Institute, as a partially purified, pelletized preparation stored in 0·05 M sodium citrate. RSV (Schmidt–Ruppin, lots 11 and 29) was supplied by University Laboratories, Highland Park, New Jersey. Viral suspensions and control materials (0·1 ml.) were inoculated subcutaneously (at the nape of the neck) into golden Syrian hamsters (Manor Farms, New York) 24–48 h after birth. Virus preparations were assayed in pock-forming units by enumeration of pocks on the chorioallantoic membrane of chick embryos using standard procedures[6].

In order to prepare RSV (Bryan), 'reconstituted' (Table 1, Group *B*), a pellet was prepared by centrifugation at 40,000*g* for 1 h followed by resuspension in Eagle's basal medium plus 5 per cent calf serum to the original volume. To prepare hamster embryo extract containing RSV (Bryan), embryos taken one day before parturition were minced and an equivalent volume of virus suspension was added. This mixture with an equal volume of 0·153 M potassium citrate (with 1·5 mg per cent hyaluronidase) was homogenized ('Virtis 45') at 4° for five 1-min runs at a setting of 10 with a 20-sec rest between runs. Eight volumes of 0·153 M potassium citrate were then added. The mixture was allowed to stand at room temperature for 30 min and afterwards kept at 4° for 24 h. The mixture was sedimented (International centrifuge) for 20 min at 4° and 1,500 r.p.m. and the supernatant was removed and spun again as above. The supernatant was cleared at 18,000*g* for 1 min (Servall centrifuge). The virus was then pelletized at 40,000*g* for 1 h (Spinco *L*) at 4°. The pellet was resuspended in a volume of Eagle's basal medium plus 5 per cent calf serum equal to the original volume of virus suspension. The preparation of hamster embryo extract (without addition of virus) was similar to that already described here and used as a control inoculum (Table 1, Group *F*).

The combined results of several experiments using RSV (Bryan), 10^5 pock-forming units, are shown in Table 1, Group *B*. Since the presence of 0·05 M sodium citrate in the RSV (Bryan) was observed to be toxic to the new-born hamsters, the virus suspension was 'reconstituted' at full titre in Eagle's basal medium plus 5 per cent calf serum. Progressively growing sarcomata had been observed in 26 of the 122 animals when the experiments were terminated after 200 days. Approximately half the animals receiving 1×10^5 pock-forming units of RSV (Bryan plus hamster embryo extract) developed tumours (Group *C*). Using a dose of only 2×10^4 pock-forming units of RSV (Bryan), about 10 per cent of the animals developed tumours (Group *A*).

Of the 148 control animals either inoculated with hamster embryo extract or Eagle's basal medium or not inoculated at all, no tumours were found after 200 days (Groups *F*, *G* and *H*).

Several experiments using the Schmidt–Ruppin strain are also summarized (Groups *D*, *E*). Based on the pock-forming units of RSV administered, the Schmidt–Ruppin strain was more oncogenic for the new-born hamsters used in this investigation than was the Bryan strain.

The results for Group *B* and *C* (Table 1) suggest a slightly enhanced oncogenic activity for the RSV (Bryan) exposed to the treatment with hamster embryo extract described here. After we had observed substantial oncogenic activity in hamsters using the RSV (Bryan) samples available in our laboratory, we abandoned any further search in our system for the identity of the 'activating factor' suggested by the work of Stewart and Landon[4].

The tumours were histologically sarcomatous (Fig. 1*A*), poorly differentiated and similar to those described by

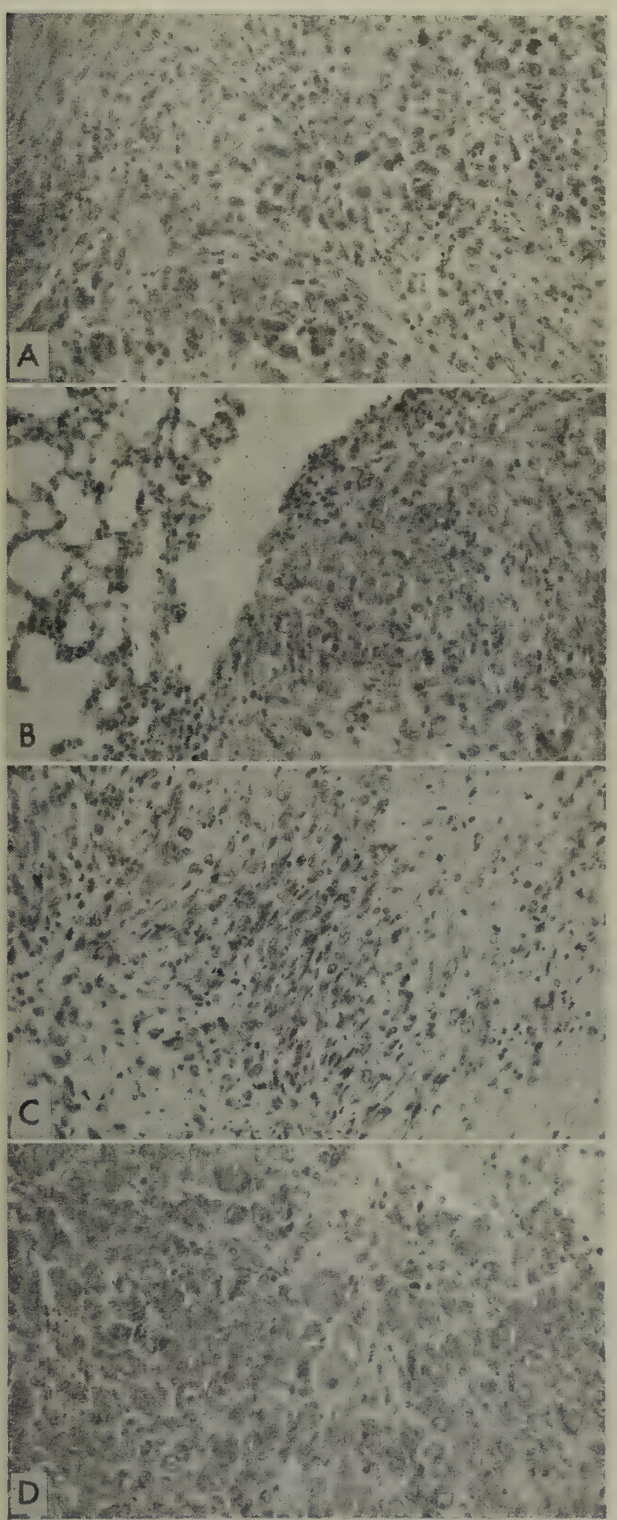

Fig. 1. Sections of tumours in hamsters inoculated at birth with Bryan strain of Rous sarcoma virus (haematoxylin–eosin stain, ×128). *A*, Poorly differentiated sarcoma. Pleomorphism; multiple nuclei; mitotic figure (lower left). Bryan *CT* 957; 2×10^4 pock-forming units diluted in Eagle's basal medium plus 5 per cent calf serum. Tumour onset: 24 days; excised after 10 days; 3·3 g. *B*, Metastasis to lung. Bryan *CT* 955, pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum. 1×10^5 pock-forming units; tumour onset 25 days; excised after 13 days; 19·9 g. *C*, Fibrosarcoma. Bryan *CT* 951; pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum; 1×10^5 pock-forming units; tumour onset 17 days; excised 37 days after onset; 3·8 g. *D*, Strap cells (in centre) suggesting rhabdomyosarcoma in the vicinity of skeletal muscle (not shown in the figure). Bryan *CT* 951, pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum. 1×10^5 pock-forming units; tumour onset 18 days; excised after 10 days; 3·2 g

Ahlstrom and Forsby[2] using the Schmidt–Ruppin strain of RSV. The latter virus strain was also used in our laboratory as a control and produced similar tumours (Groups *D* and *E*, Table 1). In a few instances, metastasis to the lungs was observed (Fig. 1*B*). In some tumours the cells were sufficiently differentiated to suggest a fibrosarcoma (Fig. 1*C*). In several cases, when the tumour was invading skeletal muscle fibres, the latter appear to have been transformed to rhabdomyosarcomatous cells (Fig. 1*D*).

The formation of rhabdomyosarcomatous cells when the tumour produced by either the Bryan or Schmidt–Ruppin strain invades skeletal muscle suggests that the virus can induce several tumour types depending on the tissue infected. This suggestion is further supported by the production of gliomata and choroid plexus papillomata following intracerebral inoculation of the Bryan virus strain into new-born hamsters[5] and the production of pleomorphic gliomata following inoculation of the Schmidt–Ruppin strain[7].

We thank Mr. Stephen Goldberg for his assistance and Drs. W. Ray Bryan, S. E. Stewart and J. C. Landon for their advice. This study was aided by grants from the National Cancer Institute (*C*-3811) and the Atomic Energy Commission *AT* (30–1)—910.

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IMPLICATIONS OF THE IMMUNOLOGICAL THEORY OF CANCER TO MULTIPLE CAUSES

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EXPERIMENTAL neoplasia has been induced in animals by radiation[1], by viruses[2,3], and by intrinsic and extrinsic chemical carcinogens. In man, too, these factors have been associated with cancer.

The evidence is still uncertain that the virus is more than a passenger in the cancer cell in man, but the lymphomatous tumour described by Burkitt[4] among children in Uganda suggests that viruses can cause some types of human cancer. Brookes and Lawley[5,6] maintain that carcinogens act by altering the nucleic acids binding to the adenine or guanine components of DNA. Gause *et al.*[7] recently described lesions in the DNA molecule, caused either by ultra-violet light or 5-fluorouracil, which they think are repaired by deoxyguanilic and deoxycytidilic acids. X-rays also can cause breaks in the DNA and RNA molecule[8]. Nowell and Hungerford[9] found loss of a normal chromosome in human myeloid leukaemia; Court Brown *et al.*[10] described the Philadelphia chromosomal abnormality in the bone marrow in myeloid leukaemia, and Upton[11] found this Philadelphia chromosome in irradiated bone marrow. So far, no consistent genotype abnormality has been found in malignant cells from fixed tissues[12], although karyotype abnormalities have been found in most cases of chronic myeloid leukaemia[13], in acute leukaemia[14] and in the bone marrow cells of patients with malignant lymphomas[15]. Witts[16] has described an association between the experience of foetal irradiation and the subsequent development of leukaemia, and Holland[17] has pointed out that patients with Down's syndrome (small acrocentric additional chromosome and mongolism) experience eighteen times the national average of leukaemia.

A low concordance for the incidence of cancer in monozygotic twins has been noted, however, which argues against chromosomal abnormality being a direct cause of carcinogenesis[18]. Recent work by Rich *et al.*[19] on virus-induced murine leukaemia suggests that aneuploidy results from, rather than initiates, the disease. The observations of Sulzberger, Sherwin and Hermann[20] suggest that a chemical could be a carcinogen in one species, and cause contact dermatitis in another. If protein binding were the sole mechanism involved in the aetiology of chemical carcinogenesis by skin painting, one would expect tumour formation in a species such as a rat or mouse where carcinogen–protein binding has been described[21]. In species which do not show protein binding such as the guinea-pig[22], the skin would be expected to be inert. Sulzberger has shown, however, that guinea-pig skin responds by developing contact dermatitis to chemicals which, in other species, cause tumour formation.

The precise site of attack of carcinogens in the chemical induction of cancer is not yet known. Green[23] suggested that carcinogen–protein complexes were formed at lipoprotein sites on the cell surface, and the resultant complexes altered the antigenic nature of the cell, which called forth an immune response from the host. The immune attack deleted, or occluded, the tissue specific antigens on the cell surface, causing a loss of cell identity. He first proposed this immunological theory of cancer in 1954 (ref. 24), when he suggested that loss of identity markers in, or on, the cell (tissue specific antigens) caused a displaced cell to be antigenetically anonymous, and that this anonymity was the fundamental cause of cancer. The maintained loss of tissue specific antigens (TSA) through subsequent cell generations may imply an alteration in the nucleic acids in all neoplasia, such as has been suggested for virus- and radiation-induced cancers. However, this is not necessarily so, as heterologous tumours have been grown for several generations in normal adult animals after having been induced under acquired tolerance[25]. These tumours re-grew in their species of origin only when induced under acquired tolerance again.

Boyden[26] has described a cytophilic antibody which is only elicited to an antigen plus Freund's complete adjuvant, and which adheres to selective cells that can then absorb the antigen. He suggests that this antibody plays a part in the reaction of delayed hypersensitivity, and is normally masked in the serum by excess of classical antibody, which competes for the antigen with that small fraction of cytophilic antibody not attached to the cells. It is thought that any antibody, or antibodies, taking part in carcinogenesis must be similarly masked by the classical responses. It is believed that, in cancer, this antibody, acting on the cell surface, deleting the tissue specific antigens, and rendering the cell anonymous, possibly also modifies the surface membranes so that the malignant cell has a metabolic advantage over normal cells.

There is evidence that malignant cells have all, or nearly all, the species and iso-antigens[27–29], so the cell surface may have been incorporating serum protein into the cell. The tumours in foreign hosts appeared to have been 'heterogenized' by binding host proteins, and this antigenic alteration was apparently maintained through several generations. It is believed that TSA-depleted cells replicate in a manner similar to these heterogenized tumours,

and that at somatic cell division the nucleus is unable to reconstitute deleted cell components.

Hamburg and Svet-Moldavsky[30] altered the antigenicity of trypsinized tumour cell suspensions by virus infection. This antigenic change prevented the tumour growing in normal mice of the strain of origin and caused enhancement in mice pre-immunized with the specific virus. Habel[31], Sjögren *et al.*[32] and Sachs[33] all reported independently on polyoma-induced tumours which could not grow in the pre-immunized strain of origin unless a very large number of tumour cells were injected. This is evidence of malignant cells incorporating a virus which partly alters the antigenic state of the cells so that an injection of a small number of heterogenized tumour cells regressed in a susceptible, pre-immunized host. An injection of a larger number, however, was able to grow and kill the host.

Loss of tissue-specific antigens in tissues undergoing carcinogenic change has been shown to take place[34–37]. If the TSA is deleted it must, presumably, leave a hitherto unexposed site open to immunological attack, and this could, at any rate theoretically, modify the antigenic nature of the cell. It is possible that a structural alteration, resultant on loss of TSA in or on the cell membranes, may also alter the functional behaviour of these membranes. Evidence has been presented to try to demonstrate this alteration in function, which is believed to be immunologically recognizable[38]. Rat spleen was sensitized to a piece of autologous 20-methylcholanthrene-induced tumour enclosed in a 'Millipore' chamber and replaced intraperitoneally. Cell suspensions were made of the autologous liver and tumour. Autologous tumour and sensitized spleen suspensions were injected subcutaneously into one flank of a guinea-pig, while the other flank received sensitized spleen and autologous liver cells. It was found that the experimental flank showed a larger flare than the control side, thus the sensitized spleen appeared to be capable of recognizing autologous malignant cells. This again suggests some antigenic alteration.

It is believed that there is a structural alteration in the membranes of a malignant cell that can be caused by factors which are endogenous or exogenous to the cell. This is supported by the work of Oppenheimer *et al.*[39], who induced sarcomata in rats to subcutaneous implants of strips of 'Cellophane', whereas Goldhaber[40] did not get sarcomata to subcutaneous 'Millipore' implants, which are porous. A mechanical alteration in the physical environment of the cell caused by an impermeable strip could possibly induce an alteration in the surfaces of adjacent cells, and initiate an immune response.

If an increased permeability to nutrients of the cytoplasmic membranes of malignant cells is postulated, the cell may be working under a metabolic advantage compared with normal cells, which, with the advance of the disease, may possibly cause gradual protein depletion of the host. This could be one of the causes of cachexia, and of the specific impairment of antibody formation, as compared with other patients, recently described in malignant disease[41].

It is possible that a variety of agents, which act on different cell components including spontaneous gene mutations[42], find their ultimate expression at the same cell sites and, by inducing an antigenic alteration on the cell surface, initiate an immune response which can culminate in malignancy.

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SELF-ADAPTIVE CONTROL AND THE RESPIRATORY SYSTEM

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IN order to design an experimental investigation of a complex biological system it is desirable, if not essential, to have a model or hypothesis which will explain the operation of the system. This is a short report of a study resulting in a hypothesis which offers a simple explanation of the normal behaviour of the entire respiratory control system. Wherever possible the hypothesis is based on structures and mechanisms known to exist.

There are many similarities between the mechanism controlling respiration and those used in the control of some engineering plants. Therefore, engineering concepts have been applied to examine the respiratory system. The main principle underlying the operation of this system is that of self-adaptive control. An adaptive control system (also called self-optimizing) operates by continuously maximizing an over-all performance index in the presence

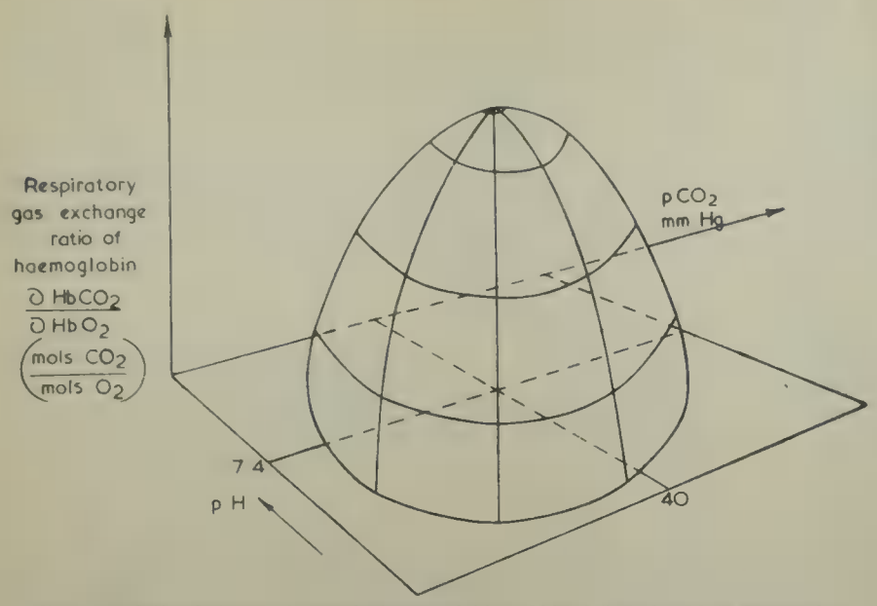


Fig. 1. Relation between the respiratory variables of blood (based on data from ref. 3)

of major external disturbances. This is achieved by adjusting the characteristics of the system[1].

The respiratory control system may be considered to consist of three interacting control loops. The processes associated with these loops are:

(1) *Chemical control.* The process by which for any given metabolic exchange requirement of both oxygen and carbon dioxide the level of ventilation is kept at a minimum.

(2) *Muscle control.* The process by which for any level of ventilation demanded by the blood chemistry, a pattern of activity of respiratory muscles is selected in which the average expenditure of energy is kept at a minimum.

(3) *Airway control.* The process by which for any alveolar gas exchange requirement the energy required to ventilate the dead space is kept at a minimum.

Each of these processes is co-ordinated and controlled by the over-all controller, a network of respiratory neurones in the brain. The controller predicts the activity which will keep the performance of the respiratory system at its optimum. For this prediction the controller uses information fed back during previous breaths.

Chemical Control

An examination of the physico-chemical properties of blood, in particular of haemoglobin, shows that there is an acidifying (Bohr) effect when haemoglobin is oxygenated in the absence of carbon dioxide. This effect is a function of pH and occurs in the range pH 6–9. It is maximal at pH 7·4 (ref. 2). The presence of carbon dioxide changes this effect. Above certain values of the partial pressure of carbon dioxide, pCO_2, and pH, oxygenation tends to make blood more alkaline[3]. The presence of carbon dioxide also increases the buffer power towards addition of fixed acid or fixed base. The relation between the function of haemoglobin and the respiratory variables may be represented by the shape of a hill (Fig. 1). This shows that the maximum number of molecules of carbon dioxide that can be exchanged per molecule of oxygen occurs at pH 7·4 and a pCO_2 = 40 mm mercury, the values normally found in arterial blood. Results by Naeraa, Strange Petersen and Boye[4] suggest that the partial pressure of oxygen, pO_2, is also involved in determining the optimum operating range of haemoglobin. It is likely that at a lower pO_2 the base of the hill is smaller (Fig. 2).

Engineering processes having these characteristics are often controlled by so-called hill-climbers. These are designed to drive the operating point of the process toward some optimum position which maximizes a performance index, for example, minimum operating cost. The mechanism involved basically finds the direction to this position by measuring, for example, the slope of the hill, and uses this information to move the operating point in this direction.

A signal which can be used by the body to define the operating point of the blood are the fluctuations in pCO_2 and pH in the arterial blood produced by breathing. At a constant frequency and depth of breathing these fluctuations are minimal at the optimum operating point and increase on moving away from this (Fig. 2). A theoretical analysis showed that the fluctuations in the arterial pCO_2 increased with increasing metabolic (exercise) carbon dioxide production[5]. It was suggested that these fluctuations and slower ones might have been signals involved in the control of ventilation. The fluctuations in arterial pH due to breathing that have been recorded in resting man show a swing of 0·01 to 0·015 pH units[6].

A control system which will keep the blood variables at the optimum values with minimum ventilation is the biological hill-climber shown in Fig. 3. Its goal is to keep the pH–pCO_2 fluctuations at a minimum. The magnitude of the signal in the arterial blood is determined by two factors. One is the mean level of ventilation which determines the position of the operating point. The other is the perturbation due to breathing which causes the fluctuations of pH and pCO_2 in the blood. This perturba-

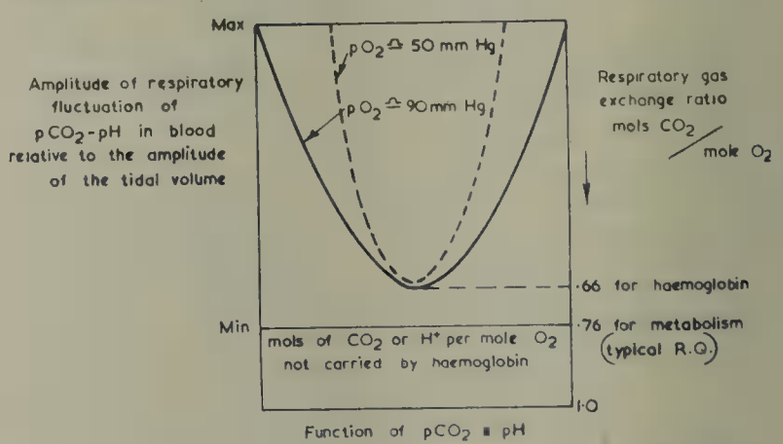

Max
Amplitude of respiratory fluctuation of pCO2-pH in blood relative to the amplitude of the tidal volume
pO2 ≙ 50 mm Hg
pO2 ≙ 90 mm Hg
Respiratory gas exchange ratio mols CO2 / mole O2
·66 for haemoglobin
·76 for metabolism (typical R.Q.)
Min
mols of CO2 or H+ per mole O2 not carried by haemoglobin
1·0
Function of pCO2 and pH

Fig. 2. The control and functional characteristics of blood

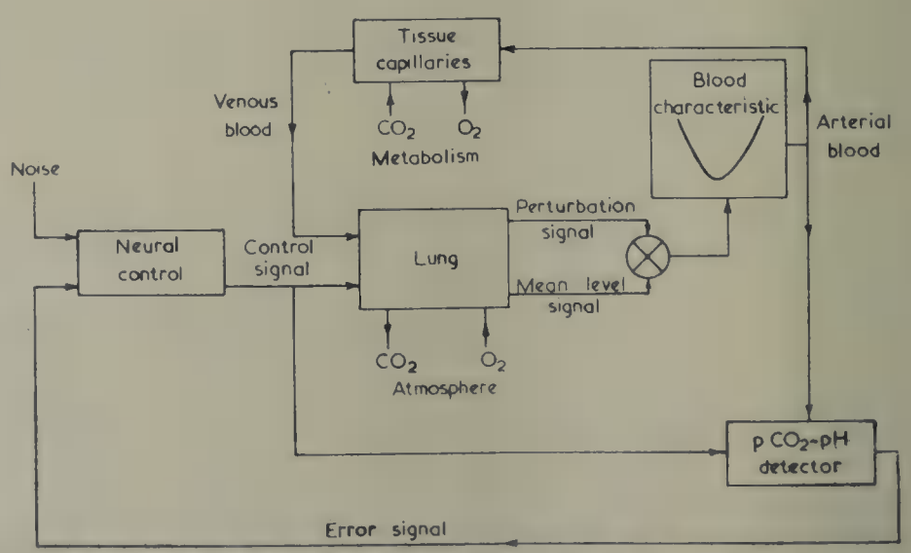


Fig. 3. Control of the respiratory gas exchange. 'Noise' is used to describe signals that are important in keeping the system operational, but which have no relevant control component

tion signal $f(pCO_2, pH)$, and a signal which is a function of the signal controlling breathing are combined in the detector to give an error signal. The control signal input to the detector is necessary to give the sign of the error signal and also to make allowance for changes in the depth and frequency of breathing. The natural choice for the detector is the area postrema. The relevant factors are its structure[7], its closeness to the medullary respiratory neurones and the relative absence of the blood-brain barrier in the area[8]. The error signal from the detector is fed back to the neural control where the control signal is corrected in the direction that will take the operating point of the blood towards the optimum. This control will keep the ventilatory gas exchange equal to that required by the metabolism of the body.

In order that this control can be effective (in particular that associated with the perturbation signal due to breathing) it is necessary that the noise-level in the mixed venous blood, of which the random fluctuation about the mean level of the pO_2 is a component, is kept at a minimum. This would be accomplished if the proportion of the cardiac output distributed to a particular region were a function of the metabolism in this region. Such control will optimize the respiratory function of the circulation. Receptors of the type found by Hughes[9], lying on the venous side of any tissues, could be part of the control mechanism which accomplishes this control. The adjustment of the peripheral circulation through the corresponding tissue would be via the autonomic nervous system.

The brain is without an oxygen store and its metabolism is essentially aerobic, so that its functioning is critically dependent on a satisfactory oxygen supply. The arterial chemo-receptors lying in the circulatory path to the brain would seem to be in the most suitable position to detect any potential lack of oxygen supply to the brain and bring about a feed-forward compensatory adjustment to the cerebral circulation and to the cardiac output generally as well as influencing respiration. This emergency type control mechanism is considered to bring about a rapid qualitative (coarse) adjustment which over-rides the optimizing control mechanism discussed.

Muscle Control

The muscles that are used for breathing may also be involved in maintaining posture or in producing movement of the trunk and limbs. Taking up a new posture or movement of limbs may result in a new pattern of breathing, for example, a new frequency of breathing and/or a new mean thoracic volume. The response usually takes a few breaths to settle down and is associated with a different spatial and temporal distribution of muscle activity.

This aspect of respiratory control involves muscle spindles and spinal cord structures. It has received considerable attention[10–12] since Nathan and Sears[13] showed its importance in normal breathing. The general arrangement of the structures is shown in Fig. 4.

The behaviour of this sub-system may be described in terms of a number of time-varying contours. One set of

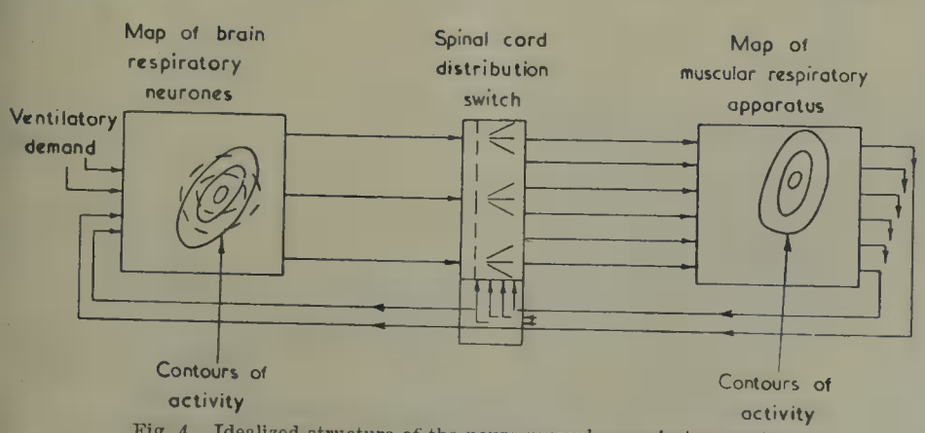


Fig. 4. Idealized structure of the neuro-muscular respiratory system

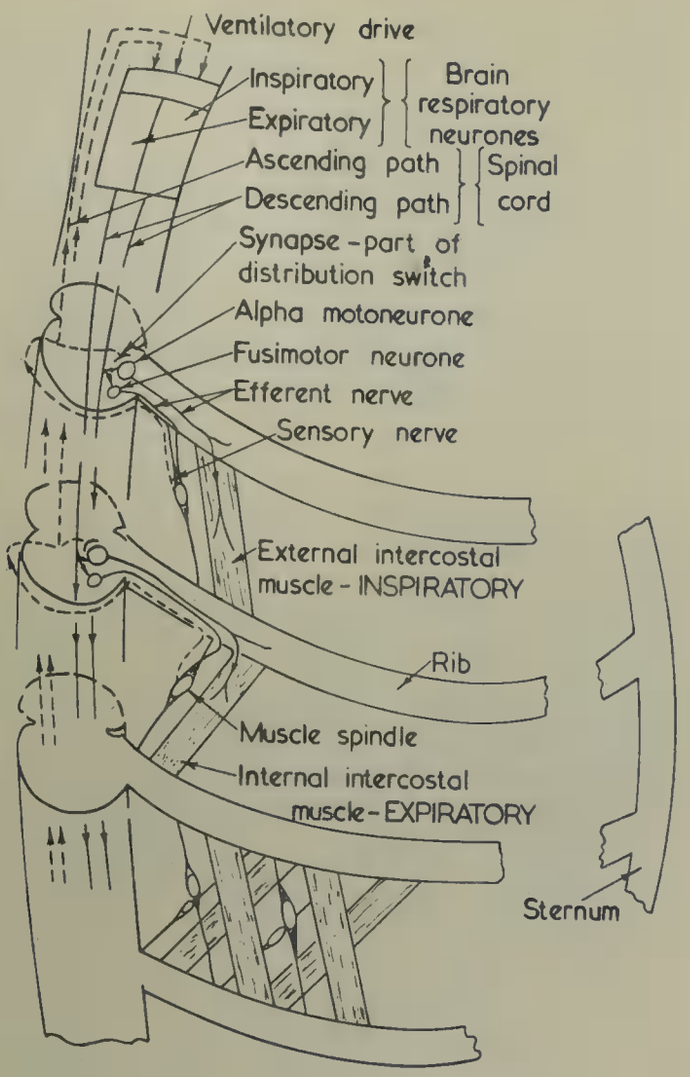


Fig. 5. Neuro-muscular control system

contours represents values of muscle activity on the grid of a muscle map (Fig. 5). The physical properties and the structure of the chest respiratory apparatus are such that progressively more energy is required for operation towards the extremities of the system. This property is represented by contours of performance index values on the muscle map. The performance index is defined in terms of the maximum number of units of ventilation obtainable per unit energy expenditure in the respiratory muscles. The contours are different for different levels of ventilation and different postures. The function of the control process is seen as the matching of the muscle activity contours with the performance index contours of highest value.

The highest level of control is the neural respiratory activity in the brain as represented by brain activity contours. The nervous activity is seen as equivalent to energy, for it is the signal determining the instantaneous energy expenditure during a breath and the duration of a breath. Once a breath has begun this control activity is not modified during natural breathing. The muscles that are activated are selected by a scanning process involving the fusimotor (γ-loop) system (Figs. 4 and 5). The scanning process selects the route of activation by a continuous appraisal of the muscle tension as determined by the muscle spindles. In this way the output from the spindles determines the spatial distribution of muscle activity. The muscles with the maximum performance index correspond to those in which the spindle output is at a minimum. This scanning process probably involves a low-amplitude 10-c/s component of muscle activity.

The process selects the optimum pattern of respiratory muscle activity by making the best

choice of inspiratory and expiratory muscles to be activated. It does this even when some of the muscles concerned with respiration are also involved in producing movement or maintaining posture.

The spindles also feed back information to the brain[14]. If the muscles shorten according to the predicted control signal then the muscle tension corresponds to the electrical activity. In this condition the ratio of the signal output from the spindles to the input to both muscles and spindles is unity and no correction of the brain activity (which determines the pattern of the next breath) is necessary. If, however, the load on the muscles has changed, the information fed back alters correspondingly. This informs the brain about the new state of the muscular system and enables it to make a new prediction of the optimum activity.

Airway Control

Temporary blocking of the vagus nerve causes an increase in the variance of the depth and frequency of breathing[15]. This implies that the energy consumption was increased for the same level of ventilation. The vagus nerve carries both the efferent fibres to the smooth muscles in the airway walls and afferent fibres coming from the receptors in the airway walls[16].

From the structure and behaviour of the respiratory system one can predict that the function of the airway control mechanism is to keep the airway diameter adjusted, by means of the smooth muscles, to those dimensions at which the forces acting on the walls of the airway due to the main muscles, are minimal. The brain activity and corresponding activity in the vagus nerve are seen as determining the size of the airway diameter at any instant relative to the activity of the main respiratory muscles. The purpose of the airway control process is to attain the condition of the airways in which the energy required to both ventilate the dead space and overcome airway resistance is minimal. The process controlling this optimization must, therefore, interact with the chemical control and muscular control mechanism.

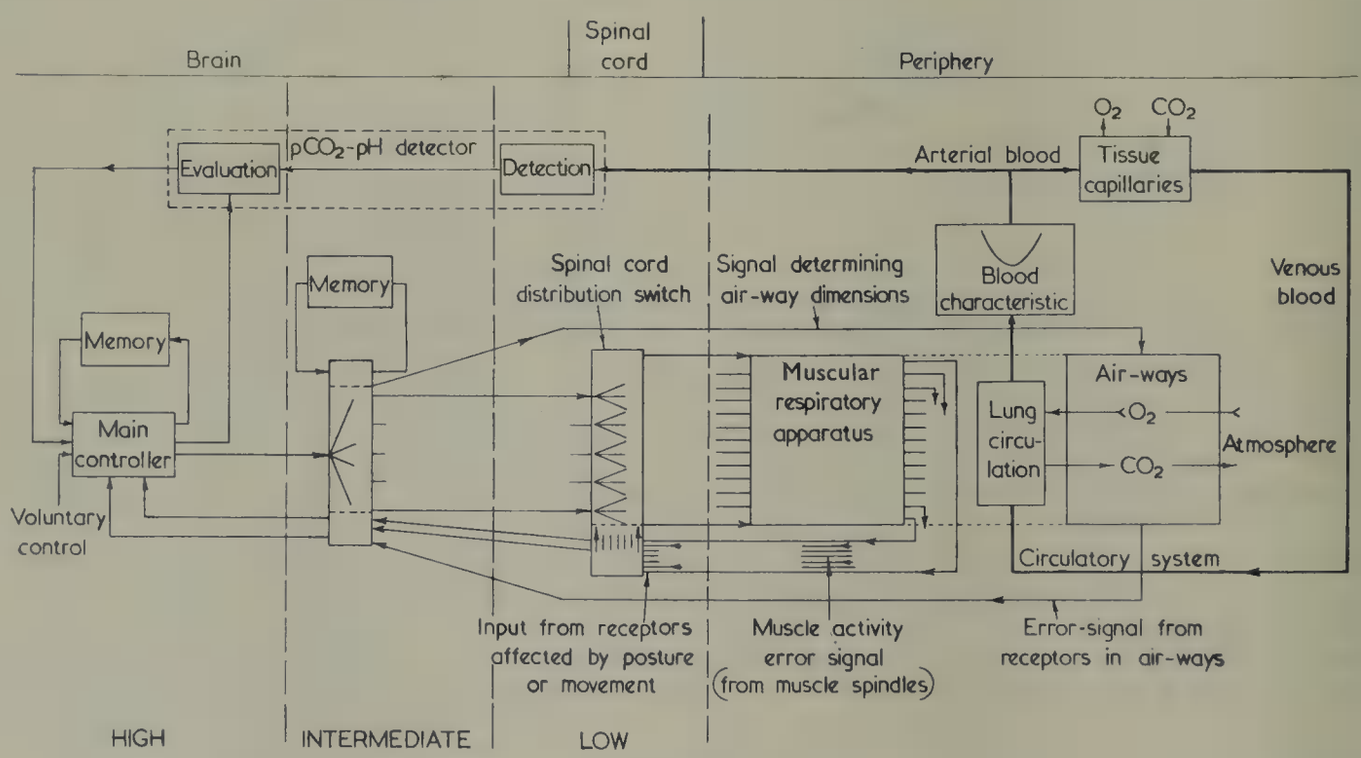
Over-all Control of Respiration

The function of the over-all control process is the combination of the functions of the three sub-systems—that is, to regulate respiration so that this is accomplished with the minimum consumption of energy. The control ensures that the ventilatory gas exchange and the metabolic gas exchange are equal.

The control action of the entire system is seen as being divided according to their importance into three levels of control (Fig. 6). A high level defines the total amount of energy, E, to be expended in a breath and the time, τ, over which it is to be dissipated. It does this by evaluating the optimal predicted activity of a breath with the actual activity in that breath. The result is the prediction of the next breath in terms of energy and time.

The optimal prediction by the high-level controller is the activity that should keep the operating point of the blood at the optimum while using a minimum of energy. The prediction is conditioned by the response of the system during previous breaths.

An intermediate-level controller interprets the orders from the higher level and produces a more detailed temporal distribution of the pattern of activity. It also produces some spatial resolution of activity. A low-level



High	Intermediate	Low
Prediction of the total energy, E, to be expended in the next breath in terms of duration, τ, tidal volume and airway diameter.	→ Increase in resolution of E in time τ and in coarse resolution of E in space.	→ Detailed resolution of E in space
← Data reduction and storage.	← Assessment in time of muscle activity accomplished	← Assessment in space of muscle activity accomplished
Assessment of operating point on blood characteristic	←	Detection of amplitude of signal $f(pCO_2, pH)$

Fig. 6. Organization of structure and function of the over-all respiratory control system

controller accomplishes the fine spatial resolution of activity. This resolution has also a small temporal component.

Consider a control sequence: a change in the operating point of blood may be the result of a change in: (1) ventilation; (2) metabolism; (3) efficiency of airway control. The chemical detector mechanism effectively determines both the magnitude and the direction of the operating point of blood from the optimum value. It does this by comparing the chemical state of the blood in the last few (probably two) breaths with that in earlier breaths. The purpose of the comparison is to decide whether or not a change of respiratory energy dissipation is required to change ventilation.

In order to resolve ventilation into tidal volume, V_t, and breath duration, τ, the main controller uses the previous prediction, that is, the memory of the activity controlling the present breath and the error signals fed back from the muscular sub-system and airway subsystem. These error signals contain the information about the mismatch in performance with respect to the best input to the sub-system. These data are reduced at the low and intermediate level controllers so that only the significant information about the accuracy of the prediction is fed back to the main controller.

The prediction and relative adjustment of the values of V_t and τ at which the mean value of E is at a minimum (for a given ventilatory demand) involve information passing around the control loops in the three sub-systems. That such a control process exists is suggested from an analysis of small variations in V_t and τ which have been observed during breathing[17]. The values of V_t and τ are positively correlated. The average cycle time for these variations is 3·6 breaths, while the cycle time expected from a random process is 3 breaths. The shortest cycle time of a search process of a controlled system of the type discussed in which there are delays and which is subjected to random disturbances would be expected to have a length of 3–4 breaths.

The function of any sub-system or component of the respiratory system can be represented by contours having values of a functional index, as was done for the respiratory muscles. The index contours of many, if not all, structures take the form of a hill. The over-all purpose of the control is to regulate the different functions so as to obtain coincidence of the hills representing the different components. In other words the individual component systems are adjusted so that these operate at their optima. By combining the hills representing different structures the map of functional index contours of the entire system is obtained. In this context the over-all control should be seen as a hill-climbing process involving an over-all functional hill of the respiratory system.

The nervous activity associated with a conscious voluntary effort is seen as over-riding the self-optimizing control process for a short period. Similar nervous activity is thought to produce the sharp rise in ventilation at the beginning of exercise[18]. The immediate response would seem to be a very qualitative increase in tidal volume (or E). This may be seen as a feed-forward signal anticipating an increase in ventilatory requirement. The adjustment of the total level of ventilation V_t and τ to the optimum values can then occur in the usual time. The selection of the optimal pattern of respiration for a new posture or during movement also involves the spinal mechanisms and muscle receptors (Fig. 6). This aspect is more complex than has been implied here. More details about the structures and mechanisms involved have been discussed by C. von Euler[11].

Only the essential points have been presented here to describe a new way of thinking about the organization and mode of operation of the respiratory control system. The underlying principle of operation, the maximization of an average functional index, may be applied to the study of most complex systems, even to the entire living organism. Certain of the aspects of respiratory control are discussed elsewhere in more detail and in relation to new experimental findings[19]. The relevance of this type of investigation to understanding such aspects of respiratory impairment as those producing the sensation of breathlessness are also discussed elsewhere[20].

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REACTION OF HUMAN LYMPHOCYTES IN CULTURE TO COMPONENTS OF THE MEDIUM

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THE *in vitro* lymphocyte transformation reaction has already found many applications[1,2]. In the hands of Bain, Vas and Lowenstein[3], and Bach and Hirschhorn[4] it has been shown to be potentially of use for histocompatibility matching in human beings. However, in order to be able to discriminate between members of a family when choosing a suitable donor for tissue transplantation, fine degrees of difference in stimulation of lymphocytes must be capable of detection.

Early in the development of this system for human histocompatibility testing, using a modification of Hirschhorn's technique[5], it was found that an unexpectedly

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Table 1. Reactions of Lymphocytes to Medium

Additions to Eagle's medium	% Large cells plus mitoses at 7 days R.H.	C.C.	E.O.	E.R.	R.W.	D.C.
Foetal calf serum + pen. and strep.	29·6	24·8	18·9	19·0	27·8	22·6
Foetal calf serum alone	—	20·5	16·2	—	31·1	15·7
aγ-Calf serum + pen. and strep.	16·6	—	—	6·9	16·9	6·8
Autologous serum + pen. and strep.	6·8	4·6	5·9	5·5	6·6	2·3
Autologous serum alone	—	2·0	5·3	3·4	3·7	1·9

large number of blastoid cells and mitoses occurred in the control culture tubes, that is, those tubes in which the lymphocytes of one individual were cultured alone in the medium for 7 days. The present experiments were designed to investigate the hypothesis that these 'high control counts' might represent a primary immune response to various constituents of the medium.

The semi-purified lymphocytes used in these experiments were prepared from 50 ml. of venous blood drawn into a syringe containing 3 ml. of heparin (1,000 units/ml.). Gelatine ('Plasmagel'[6]) was added in the proportion of 1 : 2·5 of blood, and mixed in 50-ml. centrifuge tubes, inclined at 45°. After 40 min the red cells were sedimented. The supernatant, containing white cells and gelatine in addition to plasma, was transferred to 100-ml. bottles, and a knife-point of iron powder[7] added. These bottles were incubated at 37° C, with agitation, for 1 h. The plasma was then spun in 15-ml. tubes in an International Clinical Centrifuge at 400 r.p.m. for 3 min. At this speed almost all those granulocytes which had engulfed iron-particles were sedimented. The remaining cells were then obtained from the supernatant by centrifugation in new tubes at 1,850 r.p.m. for 4 min, washed with Eagle's medium, and counted. The majority of these cells were typical small lymphocytes but there was an average granulocyte contamination of 38 per cent.

The cells were grown in 110 mm × 16 mm culture tubes, tightly closed, in a medium consisting of Eagle's minimal essential medium with 20 per cent foetal calf serum, 1 per cent L-glutamine (200 mM) and 125 units penicillin and 125 γ-streptomycin/ml. For each subsequent variation in the medium there were 2 parallel tubes and also phytohaemagglutinin ('Difco *M*') controls.

The cells were developed after 7 days by treatment with 1 per cent sodium citrate solution (to separate the clumps of cells) and fixation in 25 per cent glacial acetic acid in absolute ethyl alcohol. A minimum of 1,000 cells, fixed on slides and stained by Jenner–Giemsa, were counted microscopically by a standardized technique. One person counted the whole of one experiment without knowing the identification of the slides. The percentage of large cells plus the percentage of mitoses were added to give the 'percentage of change'. The final result was the average of the counts of 2 parallel tubes.

A few of the large cells possessing large, pale nuclei with two or three nucleoli had a grey cytoplasm which differed from the densely basophilic cytoplasm of the typical lymphoblasts. These cells were considered to be macrophages. Some other cells could not be put into either category. Therefore, all these cells with large nuclei which were clearly of a different size and type from those of the small lymphocytes were counted together as 'large cells'. The great majority of them, however, were typical lymphoblasts.

In the first 30 normal individuals it was found that the percentage of change in control tubes containing the total medium ranged from 5·0 to 48·0 per cent, with an average of 17·2 per cent.

This variable but high count in the controls was at first ascribed to the granulocyte contamination. However, the percentage of enlargement bore no relation to the percentage of granulocytes in each lymphocyte preparation. Even a 99 per cent pure lymphocyte preparation from peripheral blood gave an 11 per cent change when grown alone in the medium.

Therefore, the possibility that the 'high control counts' represented a primary immune response to various constituents of the medium was tested by deleting these constituents in a step-wise fashion. The foetal calf serum was replaced by new-born calf serum from which the γ-globulins had been removed[8] or autologous human serum (prepared from fresh clotted blood), and the penicillin and streptomycin mixture was also removed. The resultant step-wise reduction in counts in six different individuals is shown in Table 1.

Except in two instances where duplicates were not completed, each compartment in Table 1 represents the mean of two assays. The two assays obtained for these 23 pairs were tested to determine whether chance circumstances could account for discrepancies which existed or whether unknown factors were responsible. Separate χ^2 values on the original cell counts computed by a four-fold contingency table from 13 pairs totalled 16 (degrees of freedom = 13; $P > 0{\cdot}1$). The remaining 10 pairs each had large χ^2 values, suggesting that the assays were not true duplicates.

Therefore, in subsequent comparisons to determine the significance of the effect produced by foetal calf serum or penicillin and streptomycin, the only cell counts used were those derived from pairs where good agreement existed.

In considering the effect of the presence of foetal calf serum in the medium instead of autologous serum, 4 comparisons between 8 compartments revealed a highly significant difference ($\chi^2 = 1{,}100$; $d.f. = 4$; $P < 0{\cdot}001$). Similarly, 4 comparisons of the values in the presence and absence of penicillin and streptomycin were also significant ($\chi^2 = 52$; $d.f. = 4$; $P < 0{\cdot}001$).

The values obtained when aγ-globulin calf serum was used were among those in which less satisfactory agreement between the duplicate assays was found. Therefore, in comparing these results with those for foetal calf serum on one hand, or autologous serum on the other, the assay selected to represent the aγ-globulin serum was the one of the pair which was closest to the value with which it was being compared. This selection provided the least favourable value for demonstrating differences between the aγ-globulin serum and adjacent compartments. Despite this conservative method of analysing the data, the differences for 4 comparisons in each direction are significant (for each $\chi^2 > 120$; $d.f. = 4$; $P < 0{\cdot}001$).

At first glance the criticism might be raised that these results could be accounted for on the basis of nutrition: that foetal calf serum is a better nutrient than aγ-calf serum or autologous human serum. There are three reasons for thinking that this is not the case.

(i) The percentage of cells which are transformed by three days under the influence of phytohaemagglutin is consistently greater when the medium contains autologous serum than when it contains foetal calf serum.

(2) In other experiments in which cells sensitive to tuberculin or Candida antigen are stimulated by these antigens, the net count (the count in the stimulated culture minus the count in the controls) is greater when the medium contains autologous serum than when it contains foetal calf serum.

Both these results suggest that the autologous serum is in fact a better nutrient than is foetal calf serum.

(3) The addition of penicillin and streptomycin to the medium significantly increased the percentage of large cells and mitoses. It would seem to be more likely that these antibiotics are acting as antigens or as stimulants, than that they improve the nutritive properties of the media.

In the experiments in Table 1, a semi-purified lymphocyte preparation was used; however, if the same type of experiment was attempted using the total white cells present in the supernatant after sedimentation of the red cells with gelatine, the results were more erratic; the step-

wise reduction could not always be produced; and the spontaneous enlargement in the absence of foetal calf serum and antibiotics could not be reduced to such a low level.

It is possible that, in addition to the components of the medium, white blood cells other than small lymphocytes and granulocytes play a part in producing the 'high control count'. McFarland and Heilman[9] have recently reported their observations on living cultures which suggest that the macrophages play an important part in the transformation of lymphocytes to lymphoblasts. The preparation of the semi-purified lymphocytes used in the experiments reported here involved a stage of incubation and mild agitation with iron powder. This was followed by centrifugation at low speed. Examination of both the sediment and supernatant from this centrifugation reveals that the supernatant contains the nuclei of disrupted monocytes. Almost all the monocytes are removed from the lymphocyte preparation, not by engulfing iron and being sedimented, but by destruction. Monocytes, which are present in greater numbers in the total white blood cell preparations, may cause, or permit, a spontaneous enlargement in the cultures which is not so clearly controlled by alteration of the constituents of the medium.

A similar 'high control count' has been observed with the total white blood cells of the rat in culture. Unlike the human situation, these counts could not be reduced by subtraction of foetal calf serum or penicillin and streptomycin. These experiments have not yet been repeated with purified lymphocytes.

The time curve of the reaction of human lymphocytes to foetal calf serum shows that there is an upswing at 7–8 days. By contrast, the reactions of lymphocytes from individuals sensitive to PPD or Candida antigen, when cultured with these antigens, show their main response at five to six days. It may be that this difference in the time of development of large cells reflects the difference between a primary and secondary immunological response of the lymphocytes. If that were the case, the reaction to foetal calf serum would be a primary response.

Another observation emerges from these experiments. This is the apparent 'summation' effect produced by the addition of antigens. The impression is gained that the addition of antigens of different specificity causes additional lymphocytes to become involved in the reaction. A similar observation was made by Chapman and Dutton[10] in rabbits. The responses of lymphoid cells from immunized rabbits to the simultaneous presence of the immunizing antigen (heterologous serum protein) and allogeneic lymphoid cells was greater than the response to either alone.

These results are encouraging from the point of view of the application of this system to histocompatibility testing. It is reasonable to think, from what has already been shown, that allogeneic lymphocytes which differ from each other by two antigens of the same strength would stimulate one another to a greater extent than if they were to differ at only one of the two sites.

It is hoped that the avoidance of substances in the medium which, by themselves, will stimulate the lymphocytes to enlarge and divide will make it easier to detect minor differences in antigenicity between potential allograft donors.

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AFLATOXIN: EFFECT ON CULTURED HETEROPLOID HUMAN EMBRYONIC LUNG CELLS

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AFLATOXIN, a mixture of metabolites produced by *A. flavus*, has been characterized chemically[1,15], and sensitive methods for the detection of the major aflatoxin components have been reported[4,7]. In certain sensitive animal species this group of mycotoxins is acutely toxic at low concentrations, usually showing hepatotoxicity. In addition to its acute toxicity, aflatoxin B_1 is an exceedingly potent carcinogen in rats[5] and trout[2]. In biological studies the major effort has been directed towards the development of bioassays to augment and confirm the chemical procedures of analysis. There have been few reports on the biological action of this group of toxins and only three reports on the action of aflatoxin in *in vitro* systems. Juhasz and Greczi found that low concentrations of various groundnut samples destroyed cultured calf kidney cells[9]. Smith found that the toxicant inhibited the incorporation of ^{14}C-leucine into protein in various liver preparations[13]; Legator and Withrow reported that the crude and crystallized aflatoxin preparations suppressed mitosis in cultured human diploid and heteroploid embryonic lung cells[10]. The experiments reported here were designed to expand the information concerning the biological activity of aflatoxins in cell culture. The effect of aflatoxin on cell growth, cell morphology, and the synthesis of DNA was investigated.

A heteroploid human embryonic lung cell line, *L*-132, was used. The cells were cultured in monolayer, using basal medium (Eagle) with Earle's 'BSS' and 10 per cent calf serum. Aflatoxin was added to the growth medium by one of the following procedures:

(1) The chemical was dissolved in propylene glycol and added directly to the culture medium. The amount of solvent in the culture medium never exceeded 0·1 per cent; this concentration in preliminary experiments was ten times below any detectable toxic concentration.

(2) The aflatoxin preparation was dissolved in chloroform and added to the culture vessel. The chloroform was removed under nitrogen and sterile medium was added.

Usually, to achieve maximum solubility, the uninoculated medium containing the toxicant was sonicated in a Branson '*S*-75' unit for 30 sec. After sonication the medium was inoculated with approximately 100,000 cells per c.c. of medium. The cells were incubated at 37° C in an atmosphere of 3 per cent carbon dioxide.

Cell growth in the presence of aflatoxin. To determine the effect of aflatoxin on cell growth, cells were counted by a Coulter counter, and protein and DNA were determined. An aflatoxin preparation (495) containing 15 per cent aflatoxin B_1, 9 per cent G_1 and less than 1 per cent B_2 and G_2 was used. The final concentration added to the culture vessel was based on the known concentration of B_1 and G_1 in this preparation. Up to the 48th hour after the addition of the toxicant, cells exposed to 0·05, 0·1, 0·5 and 1·0 p.p.m. increased in number; the 0·05 p.p.m. concentration differed very little from the control. There was little increase in cell numbers between 48 and 93 h at 0·05, 0·1 and 0·5 p.p.m. At 1·0 p.p.m. there was a slight decrease in cell numbers. During this same period of incubation the cells in the control divided rapidly. At the highest concentration used, 5·0 p.p.m., there was an entirely different response, since only a negligible amount of growth was found at any of the time-intervals investigated. Fig. 1 summarizes the results of this experiment.

DNA was determined according to the method of Webb and Levy[16]; protein was assayed according to the method of Lowry *et al.*[11]. The DNA and protein determinations essentially confirmed the results obtained with the cell count determination. Fig. 2 indicates the effect of 0·5 p.p.m. on protein content when the cultures were examined over an incubation period of 4–93 h.

To determine the viability of the cells after various time exposures to aflatoxin, trypan-blue was used as a vital stain. No difference was found in viability between the control and the cells exposed to the toxicant except at the concentration of 5·0 p.p.m.

Morphology and giant cell formation. The *L*-132 cells exhibited many abnormal morphological patterns including

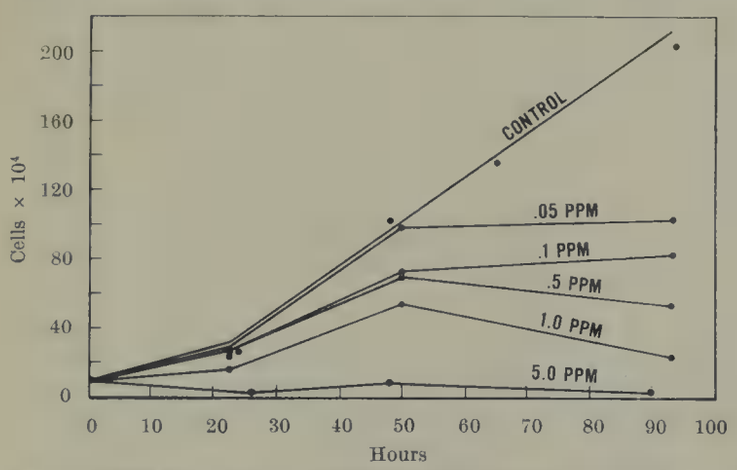

Fig. 1. Effect of aflatoxin on cell growth: cell count by Coulter counter

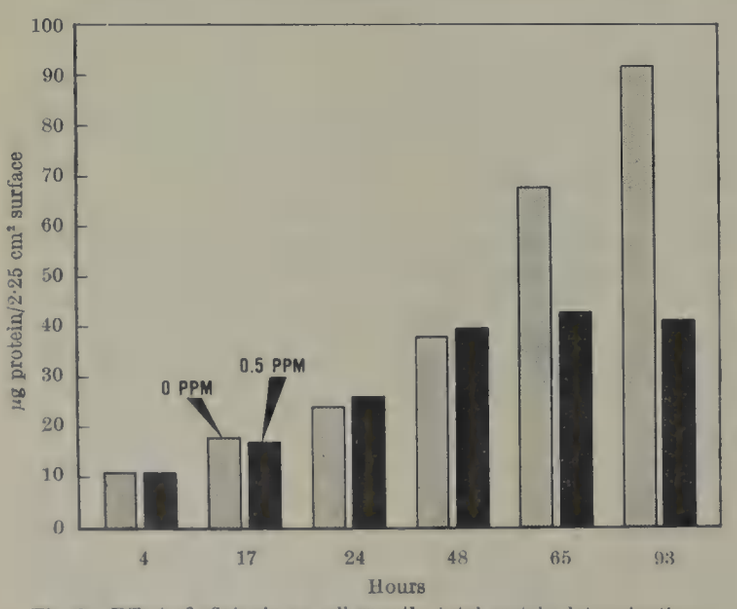

Fig. 2. Effect of aflatoxin on cell growth: total protein determination

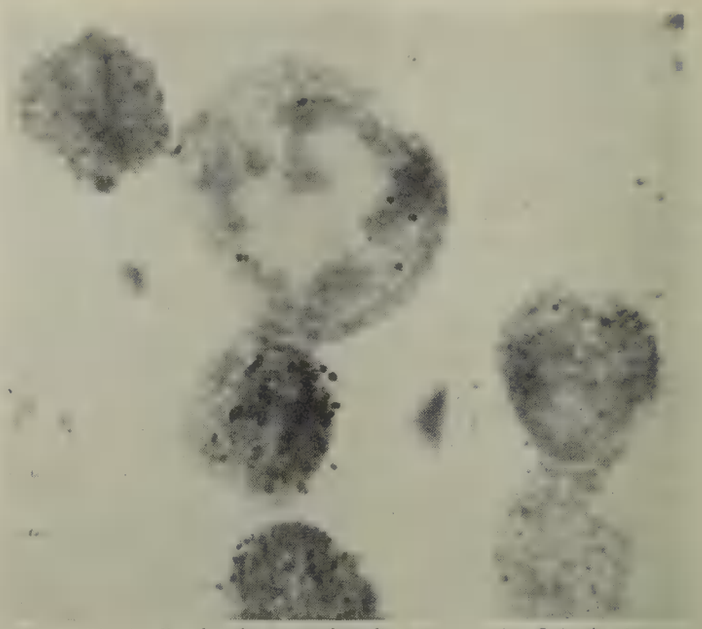

Fig. 3. Giant cells, showing vacuoles after exposure to aflatoxin, 1 p.p.m.

vacuolization and an accumulation of cellular debris. Cell vacuolization was similar to the response reported by Smith in monolayer growth of monkey kidney cells[13]. In addition there was an abnormally high number of giant cells. Fig. 3 illustrates a typical giant cell showing cell vacuolization after an exposure to 0·1 p.p.m. of aflatoxin. To determine the number of giant cells, cells were trypsinized and fixed in acetic acid–ethanol (1 : 3) and stained with 1 per cent toluidine blue. Approximately 4,000 cells were counted to determine the number of giant cells in the population. The normal population contained approximately 0·3 per cent giant cells. After an exposure of 8–12 h to 1 p.p.m. crystallized aflatoxin B_1, a 92 per cent increase in giant cells was found over the control. To determine the persistence of giant cells after exposure to the toxicant, *L*-132 cells were exposed to 1 p.p.m. of aflatoxin (495) for 24 h. After this exposure period the medium containing the toxicant was removed and fresh medium minus the toxicant was added. The number of giant cells was then determined at various time-intervals after the removal of aflatoxin. After a 24-h exposure to aflatoxin, followed by removal of the chemical, an 81–100 per cent increase in giant cells was found for a period up to 10 h. From 12 to 24 h after the removal of the toxicant, proportionately fewer giant cells were found. Table 1 shows these results.

Autoradiographic studies. To determine the effect of aflatoxin on DNA synthesis, tritiated thymidine was employed. The labelling procedure was essentially that of Moorhead and Defendi[12]. The cells were incubated in the presence of aflatoxin for 16 h before 'pulsing'. The cells were then exposed for 20 min to tritium-labelled thymidine at a concentration of 0·5 μc. per ml. The medium was removed and replaced with 50 per cent fresh medium and 50 per cent of the medium that had been removed before pulsing. After a further 12 h the cells were removed, fixed and dipped in *NTB*2 liquid emulsion (Eastman Kodak Co., Rochester, N.Y.), and the slides were incubated for 10 days before being developed.

Table 1. Giant Cell Formation after Exposure to Aflatoxin

Hours after removal of aflatoxin	Increase over control (%)
0	81
2	81
6	92
8	88
10	100
12	70
14	68
18	70
20	60
22	51
24	34

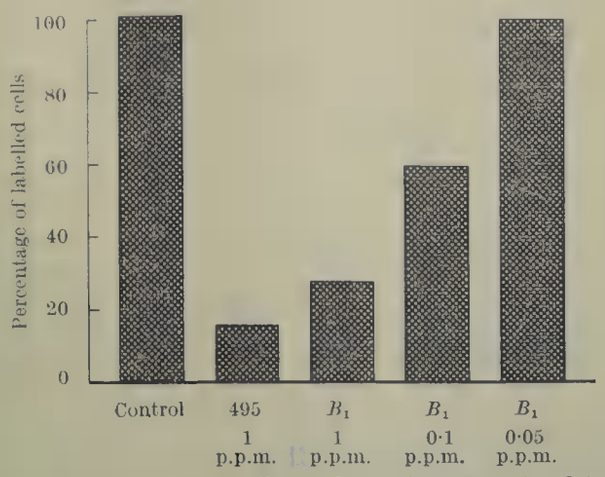

Fig. 4. Inhibition of DNA synthesis after 16 h exposure to aflatoxin

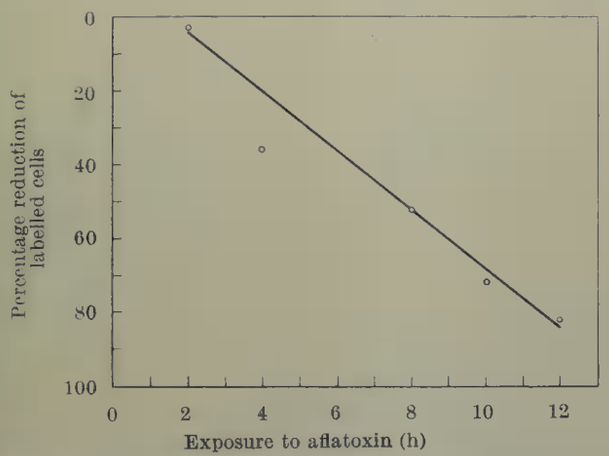

Fig. 5. Inhibition of incorporation of tritiated thymidine into DNA after exposure to aflatoxin, 1 p.p.m., for various time intervals

The number of labelled cells was determined, a minimum of 1,000 cells being counted for each determination. In this study the aflatoxin (495) and crystallized aflatoxin B_1 were used. A 40 per cent inhibition of DNA synthesis was produced by 0·1 p.p.m. of B_1 and more than 80 per cent inhibition of DNA synthesis by 1 p.p.m. of aflatoxin (495). The crude aflatoxin (495) at 1 p.p.m. was more effective than 1 p.p.m. aflatoxin B_1. The results of this experiment are summarized in Fig. 4.

Fig. 5 shows the inhibition of DNA synthesis after exposure of cells to 1 p.p.m. aflatoxin (495) for various time-intervals from 4 to 12 h. During this period, DNA synthesis was reduced by 4–84 per cent.

Discussion. A probable sequence of events for the effect of aflatoxin on this biological system can be constructed, based on the present investigations and the previous work on mitotic suppression[9]. The earliest effect of this toxicant is to suppress DNA synthesis and mitosis; this effect is detectable within the first few hours after exposure of the cells. The inhibition effect on division and DNA synthesis leads to the survival of the cell in a non-dividing stage which can be quantitated after 48 h of exposure to aflatoxin. In the static population there is no equilibrium between the lethal effects of the chemical and the number of dividing cells. This has been shown by the viability counts. The abnormal production of giant cells could well be accounted for by the enlargement of non-dividing cells. The giant cell formation seems to be of the non-syncytial type seen in tissue culture cells infected with certain viruses, and is associated with cells in which mitosis has been arrested in metaphase[3].

The suppression of mitosis, inhibition of DNA synthesis, and formation of giant cells in this system, as well as the previously reported carcinogenic action of aflatoxin, may well indicate that aflatoxin affects biological systems much as do known alkylating agents. Alkylating agents that are chemically reactive compounds are known to interact with nucleophilic (electron-rich) centres of receptor molecules of the cell[8]. Dickens and Jones have shown that aflatoxin, as well as other chemicals that contain an α–β unsaturated lactone ring, are carcinogenic[5]. It is possible that this functional group may be responsible for the overall biological effect of aflatoxin.

Alkylating agents are known to be mutagenic, carcinogenic and anti-neoplastic[6]. The carcinogenic effect may well be a manifestation of the mutagenic action[6,14]. If aflatoxin is a unique alkylating agent, the demonstration of mutagenicity and evaluation as a candidate anti-neoplastic agent may be the next steps in investigating its biological effects.

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NICOTINAMIDE MONONUCLEOTIDE PYROPHOSPHORYLASE ACTIVITY IN ASCITES CELL EXTRACTS

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NICOTINAMIDE MONONUCLEOTIDE (NMN) pyrophosphorylase activity has been detected previously in acetone powder extracts of erythrocytes[1] and ascites cells[2]. In both these cases, the *Km* for the nicotinamide was extremely high, being 0·1 M and approximately 0·1 M for the erythrocyte[1] and ascites acetone powder extracts[2], respectively. The very low affinity for nicotinamide would seem to indicate that the physiological role of this enzyme in the biosynthesis of nicotinamide adenine dinucleotide from nicotinamide is doubtful. Work in our laboratory has shown that the supernatant fluid obtained from ascites cells homogenized in a *tris*-chloride buffer contains NMN-pyrophosphorylase. The enzyme, in this crude form, has a *Km* for nicotinamide of $1{\cdot}6 \times 10^{-5}$ M and requires adenosine triphosphate (ATP) as well as 5-phosphoribosyl-1-pyrophosphate (PRPP).

Ehrlich–Lettre ascites cells were grown in Swiss albino (Webster) mice, 2–3 months of age. Cells were gathered 7–10 days after incubation and collected in heparinized vessels. Pooled cell suspensions were centrifuged at 600*g* for 5 min at room temperature and the supernatant

fluid discarded. The cells were resuspended in 6 volumes of isotonic NaCl solution and the washing procedure repeated four times to remove contamination erythrocytes. The cells were then suspended in 9 volumes of 0·05 *tris*-chloride buffer, *p*H 7·3, and homogenized for 1–2 min employing a homogenizer of the Potter–Elvehjem type. The supernatant fluid obtained by centrifuging this homogenate at 21,500*g* for 30 min served as the enzyme source.

Assay of NMN-pyrophosphorylase activity. Reaction mixture contains: 0·3 ml. of ascites supernatant, 0·7 μmoles PRPP, 2·0 μmoles ATP, 10 μmoles $MgCl_2$, 0·1 μmoles nicotinamide-^{14}C (8·0 μc./μmole) and 50 μmoles of *tris*, *p*H 7·3, in a total volume of 1 ml. Incubation was carried out at 37° C for 1 h, at which time the reaction was stopped by heating in a boiling water bath for 1½ min. The samples were then centrifuged and the supernatant material was chromatographed in paper employing the solvent *C* of Preiss and Handler[3]. The areas corresponding to NMN were cut out and counted in a liquid scintillation spectrometer as previously described[4].

The requirements for the NMN-pyrophosphorylase activity of ascites cells is shown in Table 1. Mg^{++}, PRPP and ATP are required for the complete reaction. Stimulation produced by ATP appears to be specific, not being replaced by guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP) or the di- and mono-adenine derivatives.

Identification of enzymatic product as NMN. The reaction product obtained has been characterized employing four different paper chromatographic systems: the solvent *B* and *C* of Preiss and Handler[3], isobutyrate-ammonia[5], and *N*-butanol saturated with water in an NH_4HCO_3 atmosphere[6] and paper electrophoresis. Under these conditions the unknown behaved in an identical manner to NMN. Mild alkaline hydrolysis with NH_4OH resulted in the release of a radioactive compound that behaved in an identical manner to nicotinamide in the aforementioned chromatographic and electrophoretic procedures.

A Lineweaver–Burk plot of the NMN-pyrophosphorylase activity in ascites cell supernatant employing nicotinamide as substrate is presented in Fig. 1. The *Km* for nicotinamide calculated from these data is $1{\cdot}62 \times 10^{-5}$ M.

Optimum-*p*H experiments for the enzyme under these conditions demonstrate that the enzyme has a broad peak exhibiting maximum activity from *p*H 6·5 to *p*H 9. No attempt was made to determine whether the decrease in activity at either side of the broad activity peak is reversible. However, exposure of the enzyme to a *p*H of 4·8 for a short period of time does irreversibly inhibit the enzyme.

Table 1. Requirements for the NMN-pyrophosphorylase Activity in Ascites Cells

Flask addition	NMN formed/ml. reaction mixture/h (c.p.m.)
Complete medium	96,900
−ATP	5,800
−PRPP	4,400
$-Mg^{++}$	3,700
−ATP+ADP	18,540
−ATP+AMP	5,840
−ATP+GTP	16,040
−ATP+UTP	18,580
−ATP+CTP	8,100

Reaction mixture contained 0·7 μmoles PRPP, 2·0 μmoles nucleotide, 10 μmoles $MgCl_2$, 0·1 μmoles of nicotinamide-7-^{14}C and 50 μmoles of *tris*, *p*H 7·3.

Table 2. Factors affecting NMN-pyrophosphorylase Activity in Ascites Cell Extracts

	Activity (c.p.m./ml. reaction mixture/h)
Control supernatant	113,600
Boiling H_2O bath 2 min	280
55° C for 1 min	82,246
Crude homogenate	40,620
Acetone powder extract*	1,852
Supernatant from protamine sulphate† precipitation	109,920
Stored overnight 4° C	115,250
Stored overnight −20° C	7,895
Phosphate, *p*H 7·3, 0·04 M	40,800
Pyrophosphate, *p*H 7·3, 0·03 M	2,272

* Acetone powder obtained from aliquot of ascites supernatant used in these studies. Acetone powder extracted in 9 volumes of 0·05 M *tris*, *p*H 7·3.
† 0·2 volumes of a 1 per cent protamine sulphate solution added.

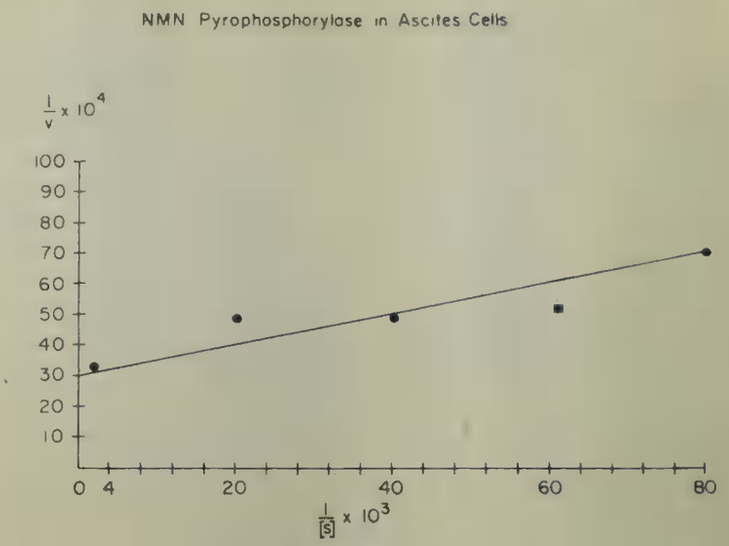

Fig. 1. Lineweaver–Burk plot of pyrophosphorylase activity employing nicotinamide as substrate

Under the condition of the assay the enzymatic activity is proportional to enzyme concentration and time of incubation. The effect of various inhibitors and treatment on the activity of NMN-pyrophosphorylase activity of ascites cells is presented in Table 2. The enzymatic activity is destroyed by boiling, freezing or exposure to cold acetone. It is inhibited by phosphate and pyrophosphate. The enzymatic activity is stable to storage at 4° C overnight and 55° C for 1 min. No activity was lost during protamine sulphate treatment of the supernatant. The activity of whole homogenate is considerably less.

The presence of an enzyme capable of converting nicotinamide to NMN in the presence of PRPP and ATP with a *Km* for nicotinamide of $1{\cdot}6 \times 10^{-5}$ M is significant and demonstrates that these cell types possess the enzymatic capacity to convert nicotinamide directly to NAD without necessitating deamidation of nicotinamide to nicotinic acid[7] and the subsequent utilization of the Preiss–Handler pathway[3] to NAD. The ascites cells studied contain endogenous nicotinamide levels of approximately 4 mμmoles/g wet weight (unpublished data). Thus, it is feasible that this reaction could, in the absence of inhibitors, proceed at a significant rate in intact cells.

The marked stimulation of NMN-pyrophosphorylase activity by ATP is similar to that observed for nicotinic acid mononucleotide pyrophosphorylase activity[8]. Whether the function of ATP is to control the direction of the reaction, as has been recently demonstrated by Nakamura *et al.*[9] for nicotinic acid mononucleotide pyrophosphorylase, remains to be determined.

Evidence that NMN-pyrophosphorylase is not the same enzyme that catalyses the conversion of nicotinic acid to nicotinic acid mononucleotide is demonstrated by the fact that NMN-pyrophosphorylase is irreversibly inactivated at *p*H 4·8 while the nicotinic acid mononucleotide pyrophosphorylase activity of the extract remains unaltered.

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MATURATION *IN VITRO* OF MOUSE, SHEEP, COW, PIG, RHESUS MONKEY AND HUMAN OVARIAN OOCYTES

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THE investigation of early development in many mammalian species is restricted by the difficulty of obtaining sufficient numbers of oocytes and embryos at particular stages of development. Post-dictyate ovarian oocytes are usually obtained after stimulation by luteinizing hormone (LH) either during the female cycle or after the injection of exogenous hormone, and ovulated eggs can be obtained in relatively large numbers after superovulation treatments. While these methods are highly satisfactory for many types of investigation, they are obviously unsuitable in the examination of species such as primates or man where the effect of exogenous hormones is undesirable or inadequately understood and in experiments demanding very large numbers of oocytes. An alternative technique has been reported in rabbits[1] and rodents[2], namely, to liberate the oocyte from its follicle into a culture medium whereon the prolonged dictyate stage is abruptly terminated, the germinal vesicle breaks down, and meiosis is resumed at diplotene/diakinesis within 2–3 h. But, with dog, primate and human oocytes, the germinal vesicles persisted after 20 h of culture[2]. The results of the present work indicate, however, that in many if not all mammalian species the culture of liberated oocytes for periods longer than 20 h will induce a resumption of meiosis at a rate exactly comparable to that occurring *in vitro* after an injection of LH into the mother. With this technique it should eventually be possible to study pre-ovulatory development, and perhaps fertilization and pre-implantation development in various species including man.

Maturation of Oocytes *in vitro*

Oocytes from mice, pigs, cows, sheep, monkey and man were liberated from their follicles into a culture medium containing heparin to prevent clotting of the follicular fluid, and then cultured in various media: Waymouth's Medium *MB* 752/1 (ref. 3), Medium 199 (ref. 4), or Hanks's saline supplemented with human and/or calf serum, antibiotics, various other additives, all media being buffered with bicarbonate (*p*H 7·2) against 5 per cent carbon dioxide in air. Samples of the oocytes were examined as whole mounts stained with aceto-orcein at various intervals after liberation in order to record the presence of a germinal vesicle or the stage of meiosis. The culture techniques were designed to encourage the migration of the corona and cumulus cells from the oocytes on to the 'Falcon' plastic used for culture, for these cells make microscopy difficult and are difficult to remove enzymatically. In almost all species examined a low proportion of the oocytes possessed a polar body and were at metaphase of the second meiotic division (metaphase-II) or were pycnotic when recovered; these oocytes were usually devoid of granulosa cells and were presumably atretic[5].

Mouse. With Difco Medium 199 supplemented with 15 per cent serum, 80 per cent of the oocytes resumed meiosis (Table 1), which is in good agreement with earlier estimates[2]. The hormonal state of the mother had no detectable effect on the proportion of oocytes resuming their maturation. A high *p*H in the medium, prolonged exposure to heparin, or high concentration of antibiotics (1,000 I.U./ml.) reduced the proportions maturing, often to nil. Almost all maturing oocytes reached early anaphase of the first meiotic division (anaphase-I), their rate of maturation to this stage being similar to the rate found *in vivo* after injecting LH into the mother[6]. Some oocytes became blocked in their maturation at early anaphase-I (Fig. 1), while the remainder progressed normally to metaphase-II and extrusion of the first polar body (Table 1). The block at early anaphase-I will be discussed below.

Table 1. Maturation of Mouse Oocytes in Difco Medium 199 with 15 per cent Bovine Serum and 100 I.U. Penicillin/ml.

Duration of culture (h)	Developmental stage of oocyte: Germinal vesicle (dictyate)	Metaphase-I	Early anaphase-I	Late anaphase-I or telophase-I	Metaphase-II and first polar body
0	76	1	—	—	3*
8·5	8	7	11	—	1
10	9	5	15	6	1
12·5–13	8	—	3	—	27
15	16	—	16	—	31
18	4	—	3	—	9

* These oocytes were presumably atretic.
After priming mice with follicle-stimulating hormone preparations followed by LH-preparations, oocytes are in anaphase between 8 and 11 h after the injection of LH, polar body extrusion begins at 10 h and is virtually complete in all oocytes by 12 h after the LH injection (ref. 6).

Cow. Ovaries were obtained from two cows in the luteal and one in the follicular stage of the cycle. Nine of the oocytes were examined immediately, and each possessed a germinal vesicle. The remaining 14 oocytes were cultured and all had a polar body and were in metaphase-II when examined 31 h later. Ovulation occurs 24–36 h after injecting LH into cows[7].

Sheep. Ovaries were obtained from two sheep in their follicular phase. Ten oocytes examined immediately possessed a germinal vesicle. Six oocytes examined after 22·5 h in culture were in metaphase-I, and out of seven examined after 46 h four had a polar body and were in metaphase-II. Polar body extrusion *in vivo* requires less than 40 h after an injection of LH into the mother[8].

Pig. I am indebted to Dr. C. Polge for permission to quote these results of our joint work. In this species, a delay of 20–24 h occurs between the injection of LH into the mother and the breakdown of the germinal vesicle[9]. A similar delay *in vitro* after liberation of the oocytes would help to explain our earlier results where germinal vesicles persisted for 20 h in cultured human and primate oocytes. Pigs were killed on day 12 of their cycle, that is, when corpora lutea were still active and the numbers of Graafian follicles maximal, or on day 18, when the corpora lutea

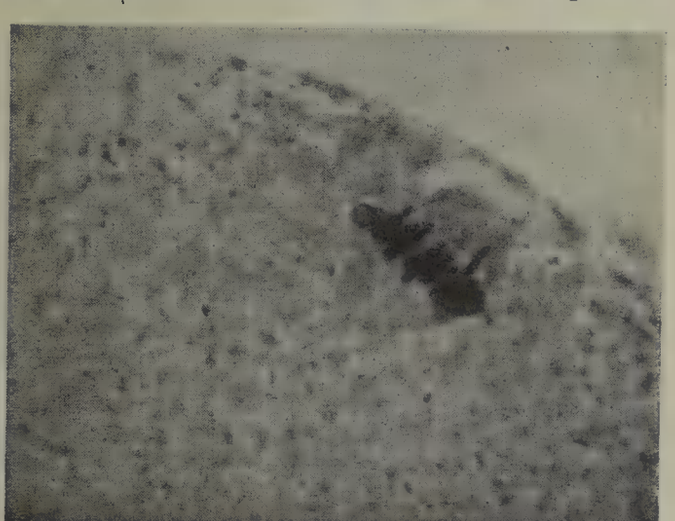

Fig. 1. Mouse oocyte blocked in its maturation at early anaphase-I. The spindle is formed and the chromosomes are extended along it. Whole mount stained in aceto-orcein. (× 1,500)

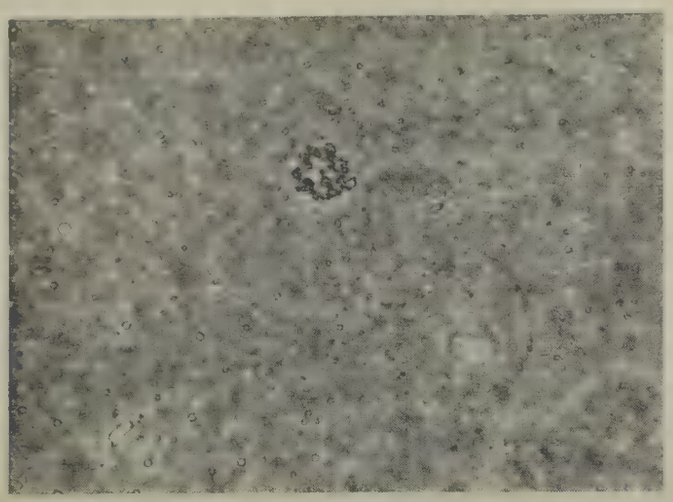

Fig. 2. Metaphase-II and first polar body (slightly out of focus in lower part of figure) in a cultured pig oocyte. Whole mount stained in aceto-orcein. (×675)

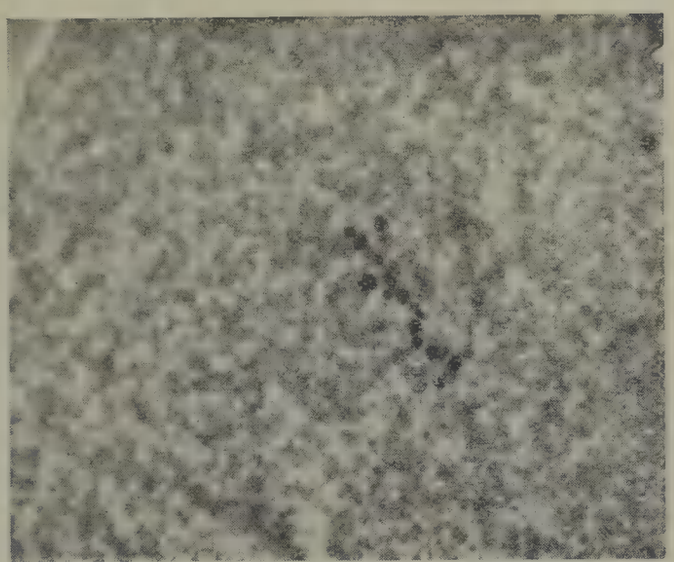

Fig. 3. Group of metaphase-II chromosomes in a human egg. Slight pressure applied after staining has brought many of the chromosomes into focus but has destroyed the outlines of the spindle. Whole mount stained in aceto-orcein. (×600)

had regressed and the wave of atresia had passed. In culture there was a delay of 20–24 h before breakdown of the germinal vesicle, and meiosis was then resumed (Table 2). Most oocytes had polar bodies, and were in metaphase-II after 43 h in culture (Fig. 2), although some were blocked at early anaphase-I. The rate of maturation was similar in oocytes from pigs killed on the 12th or 18th day of the cycle, and was closely comparable with that occurring *in vivo* during the natural cycle or after injecting LH into the mother[9].

Rhesus monkey. Ovaries from 4 monkeys were available. Polar body extrusion required approximately 30 h (Table 2). Several oocytes displayed blocking at anaphase-I, especially if grown in Burroughs Wellcome Medium 199. Ovulation occurs 24–48 h after the injection of LH into female monkeys[10].

Man. Only small pieces of ovary from patients undergoing surgery for various disorders were available, and the number of oocytes from any one piece were seldom sufficient to provide samples for examination after different periods of culture. All oocytes from one Stein–Levanthal patient failed to mature *in vitro* and have been excluded from Table 2. This might have been an example of the endocrinological state of the mother influencing the maturation of oocytes *in vitro*. Several oocytes examined after 24 h had prometaphase chromosomes. Polar body extrusion evidently required a maximum of 40–48 h (Table 2, Fig. 3). Ovulation occurs 24–48 h after the injection of LH into women[11].

Block in Development at Early Anaphase-I

The block at anaphase-I might have physiological significance, for a natural arrest of development occurs in most mammalian species at late metaphase-II just before fertilization. With Difco medium 199 supplied ready for use, between 10 and 45 per cent of mouse oocytes were blocked in replicate cultures (Table 1), whereas with Burroughs Wellcome 10 times concentrated 199 approximately 80 per cent were blocked, the great majority at early anaphase-I and a few at telophase (Table 3). In many blocked oocytes a distinct bulge from the vitellus could be seen next to the spindle after fixation. Maturation was blocked rather than delayed, for prolonging the culture period did not alter the incidence of blocked oocytes (Tables 1 and 3). Examination of the listed contents of the two media 199 revealed no differences in composition to explain why one should cause more blocking than the other, the major difference being the inclusion of Earle's saline in Burroughs Wellcome 199 as opposed to Hanks's saline in Difco 199.

Nevertheless, blocking was evidently due to deficiencies in the culture medium as shown by the following experiments. First, a sample of mouse oocytes was divided into three groups and cultured in Difco 199, Burroughs Wellcome 199, or a mixture of equal parts of the two. The incidence of blocking was profoundly influenced by

Table 3. Maturation of Mouse Oocytes in Burroughs Wellcome 199 with 15 per cent Bovine Serum and 100 i.u. Penicillin/ml.

Duration of culture (h)	Stage of development of the oocytes: Germinal vesicle (dictyate)	Meta-phase-I	Early anaphase-I	Late anaphase-I or telophase-I	Metaphase-II and first polar body
11·5–12	4	0	6	1	0
13–14	8	3	16	2	4
16–17	15	2	16	2	4
18–19	34	8	58	6	11
25	9	—	5	—	6

Table 2. Maturation of Pig, Rhesus Monkey and Human Ovarian Oocytes *in vitro*

Species	No. of animals	Duration of culture (h)	Stage of development of the oocytes: Germinal vesicle (dictyate)	Prophase-I	Metaphase-I	Early anaphase-I	Late anaphase-I or telophase-I	Metaphase-II and first polar body
Pig	8	0	20	—	—	—	—	—
		9–9·5	14	—	—	—	—	—
		17·5–20	14	3	5	—	—	—
		24–25	6	11	—	—	—	—
		29–30	10	—	5	2	—	1
		43–46	17	—	—	2	3	20
Rhesus‡	4	0	19	—	—	—	—	—
		23	10	5	5	—	—	1
		29	8	—	1	2	1	1
		41	8	—	1	4	—	2
Man	†	0	11	—	—	—	—	—
		24	7	7	—	—	—	—
		40	2	—	1	—	—	1+1?
		48	6	—	—	—	—	3+2?
		63	6	—	2?	—	—	8*

* Several degenerating.
† Pieces of ovary from several donors.
‡ Oocytes from two monkeys grown in Burroughs Wellcome 199 were blocked at early anaphase-I at 41 h.

Table 4. Influence of the Medium on the Maturation of Mouse Oocytes after 16 h in Culture

Medium*	Any extra treatment	Stage of development of the oocytes: Metaphase-I or early anaphase-I	Stage of development of the oocytes: Metaphase-II first polar body
Difco 199+15 per cent bovine serum	—	3	32
Equal parts, Difco 199+Burroughs Wellcome 199+15 per cent bovine serum	—	6	39
Burroughs Wellcome 199+15 per cent bovine serum	—	19	7
Difco 199+15 per cent bovine serum	Mice super-ovulated, oocytes recovered 2 h after HCG†	7	12
Difco 199+15 per cent bovine serum	Pig follicular fluid (33 per cent v/v)	3	5
Pig follicular fluid	—	14	5

* 100 I.U. penicillin added to all media.
† HCG, human chorionic gonadotrophin.

the culture medium (Table 4). Secondly, mice were super-ovulated with gonadotrophins[6], and 2 h after the injection of HCG their oocytes were liberated. Polar body extrusion from these oocytes would have occurred *in vivo* within 9 h. After 15 h in Difco 199 and 15 per cent calf serum, 7 of the 19 oocytes were blocked. Thirdly, even the use of neat or diluted pig follicular fluid failed to alleviate the block in mouse oocytes (Table 4).

Various attempts, so far unsuccessful, have been made to alleviate the block at anaphase-I by modifying the culture medium. Blocking could not be attributed to heparin, the source of serum or distilled water, the *p*H of the medium (although higher *p*Hs reduced the proportion slightly), or to the antibiotics in the medium. Nor has the incidence of blocked oocytes been influenced by the provision of feeder cells (*L*-strain or granulosa monolayers), addition of extra ATP, addition of extra oxidized and/or reduced glutathione in case extra disulphide or sulphydryl groups were required by the oocytes for anaphase separation[12], addition of folinic and ascorbic acids to facilitate the shedding of RNA attached to chromosomes at metaphase[13], or changing the gas phase to 5 per cent carbon dioxide in oxygen. Fertilized 1-celled mouse eggs are difficult to grow *in vitro* and the block at early anaphase might be due to similar deficiencies in the culture medium.

Experimental Studies with Oocytes maturing *in vitro*

Oocytes maturing *in vitro* are evidently not atretic. This is shown by: (1) the similarity in all species examined between the duration of maturation *in vitro* and *in vivo*; (2) the profound influence of the culture medium on the incidence of blocking an early anaphase-I; and (3) observations that matured rabbit[1] and pig[14] oocytes can be fertilized *in vivo* and that the fertilization of matured human oocytes can proceed *in vitro* at least as far as penetration of spermatozoa into the perivitelline space[15]. The technique thus promises to provide excellent material for investigating the dynamics of early mammalian development. For example, the role of the centrioles in meiosis and fertilization could be investigated using the techniques applied to the sea-urchin egg[16]; the spindle of the second maturation division is formed within 30 min of the previous telophase which implies a rapid re-organization of the centrioles. Distinct asters with astral fibres attached to the nearest point on the egg membrane could be seen in several mouse oocytes at the first and second meiotic division, and this eccentric attachment of the fibres to the membrane could account for spindle rotation at telophase and the smallness of the polar bodies. Chromosome mechanics at meiosis could also be examined, especially with reference to meiotic anomalies leading to trisomy in oocytes from older women[2].

The technique also offers the opportunity of investigating early development in species difficult to study otherwise, for example, primates, and of obtaining many oocytes and increased numbers of progeny from valuable livestock by egg transfer. Large numbers of oocytes can be handled in culture: we have handled up to 700 pig oocytes on several collections without undue difficulty[14]. With these numbers it should be possible to apply biochemical analyses to mammalian eggs similar to those applied to invertebrate and amphibian eggs. Ribosomal RNA is synthesized during the pre-ovulatory and pre-neurula stages in amphibians, *s*RNA and *m*RNA synthesis increasing considerably during late cleavage[17]. A similar situation might hold in mammals, where increasing amounts of RNA are synthesized after the second cleavage[18]. Several phenomena occurring in the mammalian morula might be related to the initiation of *m*RNA synthesis at this stage, for example, the initiation of heterochromatin in the sex chromosomes and sex chromatin in the nucleus[19].

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PROCESSES OF EXCITATION AND INHIBITION IN SINGLE MECHANORECEPTORS (PACINIAN CORPUSCLES)

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INVESTIGATING various properties of Pacinian corpuscles we found that in a number of cases their electrical response contains positive deviations of potential, the origin of which is impossible to understand within the limits of present concepts of their activity[1–4]. Some peculiarities of this phenomenon are discussed in the present article. A detailed description of the experimental technique has been presented elsewhere[5–8].

Fig. 1 demonstrates gradual reaction of the receptor elicited by mechanical shocks of triangular form. It is seen that with increase in duration of the stimulus the response declined in amplitude, decreased in rate of rise

and increased in duration. In some records[2–5] a little hyperpolarization of gradual character appears. It may be noticed also that the appearance of depolarization is associated only with the effect of the posterior front of the stimulus. Its anterior front either produces no changes or gives rise to positive deviations of the potential. The diminution in the amplitude and in the rate of rise of the gradual response of the receptor with the decrease of the velocity of displacement has already been described[9].

It is well known[10] that the capsule of the receptor, while strongly attenuating the static component of the stimulus, conducts its dynamic component well. The prolongation of the triangular stimulus is actually equal to the increase in duration of the dynamic component of such stimulation. Therefore the observed increase in the duration of the gradual response means that the receiving elements of the mechano-sensitive membrane of the Pacinian corpuscles adapt themselves to the long-lasting stimulus much more slowly than was previously thought (see also refs. 11 and 12). The fact that the reactions of the receptor to the ascending and descending parts of the mechanical shock differ is more difficult to understand, for it is well known[5,8,9] that responses of the intact corpuscles to the on- and off-stimuli are more or less identical. Furthermore, it is now clear[8] that mechanical shock produces oscillations in the capsule of the receptor which, provided the stimulation is sufficiently long, induce off-reaction, that is, the mechanism of generation of off-responses is actually the same as that of on-responses. If the amplitude of mechanical stimulus is sufficiently large these oscillations evoke multiple responses of the receptor (see also Fig. 2, 11).

The systematic study of a great number of receptors showed that, in practice, depolarization of the nerve ending is always accompanied by hyperpolarization phenomena and not infrequently the reactions to anterior and posterior fronts of the stimulation may be essentially different especially when long-lasting triangular (sinusoidal) mechanical shocks are applied. Records presented in Fig. 2 illustrate the phenomena in question. It can be seen that the amplitude, rate of rise, duration and shape of the positive response of the receptor vary both from experiment to experiment and in the same experiment. The appearance of hyperpolarization is in no way connected with the moment of the spike origination and is observed even in its absence. However, positive deviations of the potential are most clearly observed on the background of the preceding depolarization. The character of the reaction is to a great extent determined by the amplitude and duration of the mechanical shock as well as by the site of its application, etc.

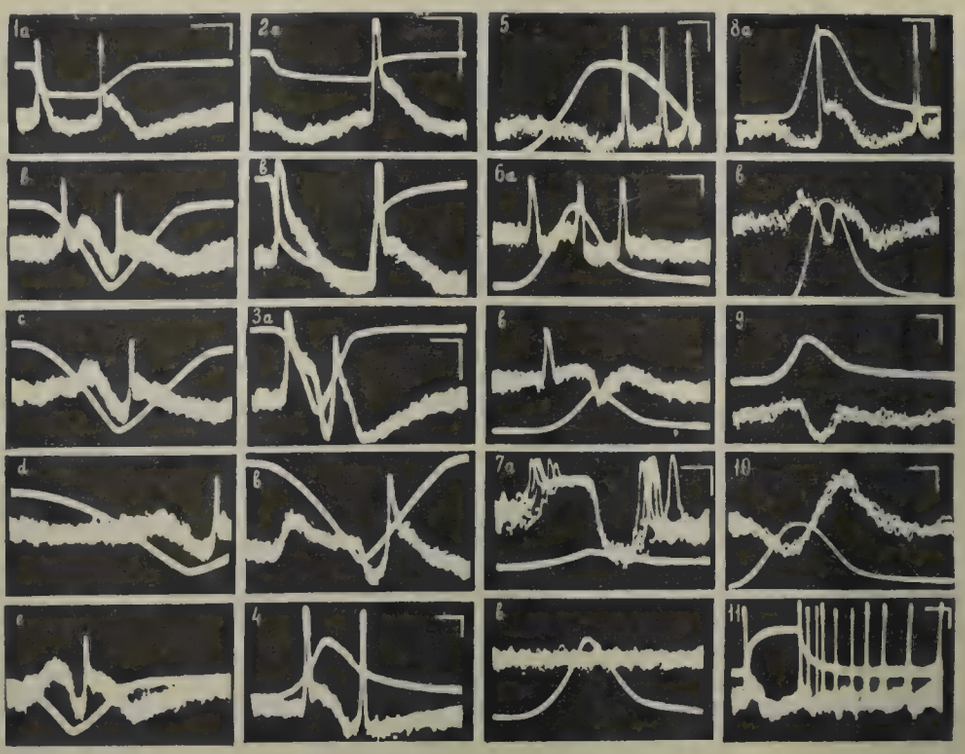

Fig. 2. Response of the receptor under different conditions of stimulation (11 different Pacinian corpuscles). In 6*b*, amplification in the system of recording the mechanical shock (lower beam) is diminished in relation to 6*a* and the direction of the shock is changed. In 7*b*, the receptor is killed and the absence of mechanical artefact in the system of recording under stimulation greater than in 7*a* is demonstrated. In 8*b* the direction of shock is changed as compared to 8*a*. Peak potentials in 8*b*, 9, 10 are blocked by procaine. The appearance of depolarization responses in almost all records in switching off the stimulation is evidently connected with the origin of oscillation process in the capsule of the receptor (see record 11); time mark: 1, 3, 5·0 msec; 2, 5, 8, 10, 11, 4·0 msec; 4, 3·0 msec; 6, 7, 9, 2·0 msec; calibration: 1, 3, 4, 5, 6, 7, 8, 11, 20·0 μV; 2, 30·0 μV; 9, 10, 15·0 μV

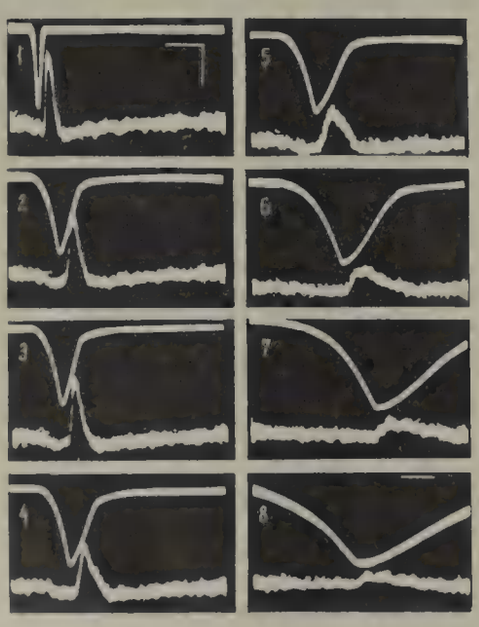

Fig. 1. Changes in the response of mechanoreceptor (lower beam) caused by alterations in duration of mechanical stimulation (upper beam). Peak potentials are blocked by procaine. Calibration 50·0 μV; time mark (msec): 1–7, 5·0; 8, 10·0

It is known[13] that the nerve ending in the Pacinian corpuscle has the shape of an elliptic cylinder. Hubbard[10] suggested that the ratio between the great (a) and small (b) transverse axes of the nerve ending is increased by stimulation. Consequently the surface of the receptor membrane increases, which results in excitation.

However, the increase in the ratio $a : b$ occurs only if the mechanical stimulus is applied along the axis 'b'. The stimulus directed along the axis 'a' decreases the ratio $a : b$ and consequently decreases the surface of the receptor membrane. Suppose that depolarization of the nerve ending membrane is caused by an increase (distension) and hyperpolarization by a decrease (constriction) in the surface of the latter. Then stimulation applied along the axis 'b' would produce depolarization, while that falling in the direction of the axis 'a' would evoke hyperpolarization. In experimental verification of this assumption possible appearance of oscillation processes in the capsule of the receptor had to be taken into consideration[8]. In this connexion short-lasting shocks with considerable amplitude and rate of rise were not to be used, as the phenomena of hyperpolarization would have been masked by arising depolarizing responses.

In Fig. 3 are shown oscillograms which demonstrate a good agreement between expected and experimental data. It is obvious that with rotation of the corpuscle around its longitudinal axis the anterior front of the long-

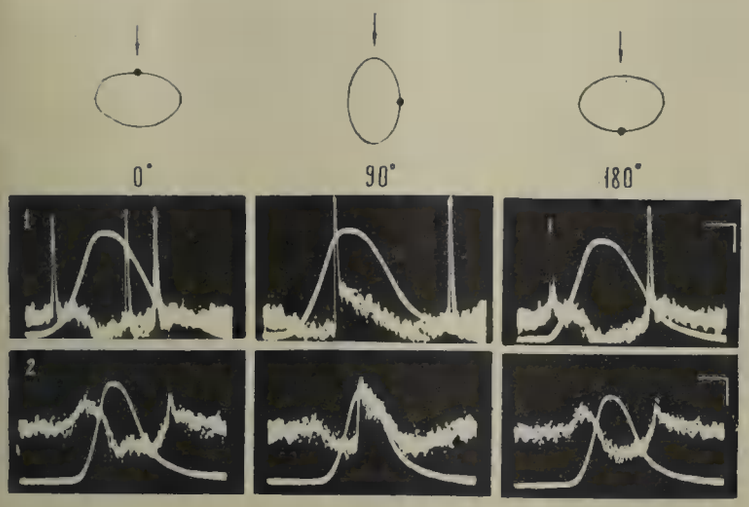

Fig. 3. Functional asymmetry of the receptor. Top: scheme of supposed correlation between the direction of stimulus and position of the nerve ending (its small transverse axis). Bottom: corresponding schemes of responses of Pacinian corpuscles in two different experiments. In 2 spikes blocked by procaine; time mark (msec): 1 and 2, 4·0; calibration (μV): 1, 10·0; 2, 5·0

lasting stimulus which is responsible for depolarizing reaction produces hyperpolarization response and vice versa. It should be noted that reverse-directed deformation due to elastic properties of the capsule at the moment of cessation of stimulation arises[8] and therefore the posterior front of the stimulus acts contrary to the anterior one.

On the basis of the data obtained, the responses of Pacinian corpuscles presented in Figs. 1 and 2 may be readily explained (a detailed consideration of the hyperpolarization phenomenon will be presented elsewhere). Positive deviations of potentials are usually accompanied by some decrease in the receptor excitability (Fig. 2, cf. 2*a* and 2*b*); therefore, on the analogy with inhibitory synaptic potentials, they may be referred to as inhibitory receptor potentials (IRP), while the negative changes in the potential may be called excitatory receptor potentials (ERP).

In view of the data obtained the participation of mediators in the processes of excitation of Pacinian corpuscles[14] seems scarcely feasible.

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A NEW FLUORESCENT ANTIBODY METHOD: MIXED ANTIGLOBULIN IMMUNOFLUORESCENCE OR LABELLED ANTIGEN INDIRECT IMMUNOFLUORESCENCE STAINING

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INVESTIGATIONS by Coombs *et al.* on mixed agglutination[1] and mixed antiglobulin tests[2] have revealed that an antibody combined with its specific antigen on a cell may have a free antibody combining site capable of reacting with another molecule of its specific antigen. In the mixed agglutination tests these free combining sites are detected with antigens (immunoglobulins for the mixed antiglobulin tests) attached to red cell indicators. We have investigated the use of antigens (immunoglobulins) labelled with fluorescein[3,4] in a similar system using frozen tissue sections[3] as primary antigens. This method may be referred to as mixed antiglobulin immunofluorescence (IF) or mixed immunofluorescence (MIF). Unfortunately the latter term may be confused with the 'mixture of colours'[5] obtained by labelling with different fluorochromes, so that the cumbersome but more accurate term 'labelled antigen indirect IF staining' may be preferable to describe the method. For the sake of clarity the established IIF (indirect immunofluorescent) staining procedure[6] may now be referred to as the 'labelled antiglobulin indirect IF staining' in contradistinction to the 'labelled antigen indirect IF' or MIF method.

Fig. 1 provides a diagrammatic comparison of the principles involved in labelled antiglobulin and labelled antigen indirect IF staining. Table 1 summarizes the specific techniques employed in the labelled antigen or MIF staining method.

Serial dilutions of human sera containing tissue antibodies were tested on frozen sections of tissue cut in a cryostat[3]. Labelled immunoglobulins were prepared by conjugating either commercial human serum fraction *F*II or *IgG* prepared by DEAE chromatography. For conjugation, 0·08 mg FITC/mg protein was found to yield satisfactory reagents (FITC, fluorescein isothiocyanate, was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio). Both the antiglobulin (step 3) and the normal rabbit serum used as the diluent (after 1 : 15 dilution in buffered saline) of the labelled antigen (step 5) were heat-inactivated before dilution because some slight enhancement of MIF staining (sometimes as much as a whole dilution in the staining end-point of the human serum) could be effected thereby.

The precipitating activity of the antiglobulins and of the labelled antigens provided estimates of their respective antibody and antigen activities. A concentration of 1 unit/ml. of antiglobulin was defined as the highest dilution which would give a visible line of precipitation in a standard agar-gel diffusion precipitation test (6 wells 8 mm from a centre well, all 2·5 mm in diameter) using serial 2-fold dilutions of the antiglobulin in the peripheral wells and 1 mg/ml. of *IgG* or *F*II in the centre well. Conversely, 1 unit/ml. of labelled immunoglobulin was

Table 1. Procedure for Mixed Antiglobulin or Labelled Antigen Indirect IF

Step	Reagents	Conditions	Time (min)
1	Human sera	Serial dilutions to end-point	20
2	Wash	PO_4 buffered saline	60
3	Anti-human immunoglobulin	Heated 56° C 30 min, diluted to 1–4 units	20
4	Wash	PO_4 buffered saline	60
5	Labelled human immunoglobulin	Diluted to 1–2 units in heated normal rabbit serum (1/15 dilution)	20
6	Wash	PO_4 buffered saline	120
7	Glycerin mount	1/10 buffer: 9/10 glycerin	

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defined as the concentration present in the highest dilution yielding a visible line of precipitation with 2 units of an antiglobulin in the centre well. Most labelled preparations of *IgG* or *F*II which we have used have contained about 0·05 mg of protein/ml. in 1 unit. A more detailed report on the relation between the unitages of antiglobulins and labelled immunoglobulins and their respective reactivity in MIF staining will be made at a later date[14].

Phosphate-buffered saline[3] served as a diluent for all reagents as well as a washing solution. Prolonged washing (60 min for steps 2 and 4 and 120 min for step 6 in Table 1) was used to minimize non-specific staining. While some exploratory studies indicated that washing times of 20, 20 and 120 min (for steps 2, 4 and 6, respectively) might yield equally satisfactory results, the prolonged washing times listed in Table 1 were adhered to throughout most of these investigations to provide an added margin of safety. Fluorescence microscopy was performed as described previously[7].

Studies of MIF or labelled antigen IF staining revealed that under the conditions used non-specific staining ranged from an undetectable to a low acceptable level. The latter appeared as a dull-green background staining which could readily be distinguished from specific IF staining. In most of these investigations it was difficult to distinguish between some traces of green auto-fluorescence of tissue and traces of non-specific staining.

The appearance of specific MIF staining patterns given by tissue antibodies, such as antinuclear factor[8] (ANF), could not be distinguished qualitatively (in readings of coded preparations) from those obtained in parallel studies by the established labelled antiglobulin indirect IF staining procedure. At the quantitative level, comparisons were made by titrating human sera (usually in doubling dilutions) to IF staining end-points. An end-point was defined as the highest dilution of the patient's serum which yielded an IF staining reaction clearly distinguishable from that of negative control sera. Preparations were randomized and read as unknowns by two or more observers. Under these conditions, IF staining end-points varied by as much as plus or minus one doubling dilution.

Table 2. Titrations of Antinuclear and Antinucleolar Antibodies by the Labelled Antigen (MIF)* and Labelled Antiglobulin (IIF)* Indirect IF* Staining Methods

Case	Sera tested: Type of antibody	Sera tested: Dil. of sera	IF staining reactions: Rabbit anti-human* MIF	Rabbit anti-human* IIF	Goat anti-human* MIF	Goat anti-human* IIF
B	None	1 : 25	–	–	–	–
D	ANF*	1 : 100	+	+	+	+
		1 : 200	+	+	+	+
		1 : 400	+	+	+	+
		1 : 800	W*	W	W	W
		1 : 1,600	W	–	–	W
W	Nucleoli	1 : 25	+	+	–	+
		1 : 50	W	W	–	W

* IF, immunofluorescence. MIF, mixed (antiglobulin) IF or labelled antigen indirect IF. IIF, indirect IF or labelled antiglobulin IIF.

Rabbit and goat anti-human antisera to human *IgG*. Other antibodies could be demonstrated in these by immunoelectrophoresis but did not appear to be relevant to the MIF or the IIF staining reactions as judged by comparison with monospecific antisera to *IgG*.

ANF, antinuclear factor.

+, moderate to strong positive.

W, weak positive.

Table 2 summarizes some typical titrations performed with human sera containing two types of antibodies. Titrations were performed by the labelled antigen and labelled antiglobulin methods using both goat and rabbit antisera to human immunoglobulins. For both the MIF and IIF staining 4 units of the goat antiserum were used while the rabbit antiserum was used at dilutions containing 2 units and 1/5 units for the MIF and IIF staining, respectively. Both antiglobulins contained not only antibodies to human *IgG* (as indicated by their unitage) but also antibodies to other serum proteins as revealed by immunoelectrophoresis. For MIF staining 2 units of a labelled human immunoglobulin (*F*II, Koch Light Laboratories, Ltd., Colnbrook, Buckinghamshire) with an estimated molar fluorescein to protein ratio of 8·1/1 was used as antigen for the top layer.

The serum of patient *D*, as shown in Table 2, yielded a nuclear staining titre of 1 : 800 or 1 : 1,600 by both the MIF and the IIF staining methods under the conditions used. Essentially comparable results were obtained with the goat and rabbit antiglobulins. Similarly, the serum of patient *W*, which contained antibodies to nucleoli,

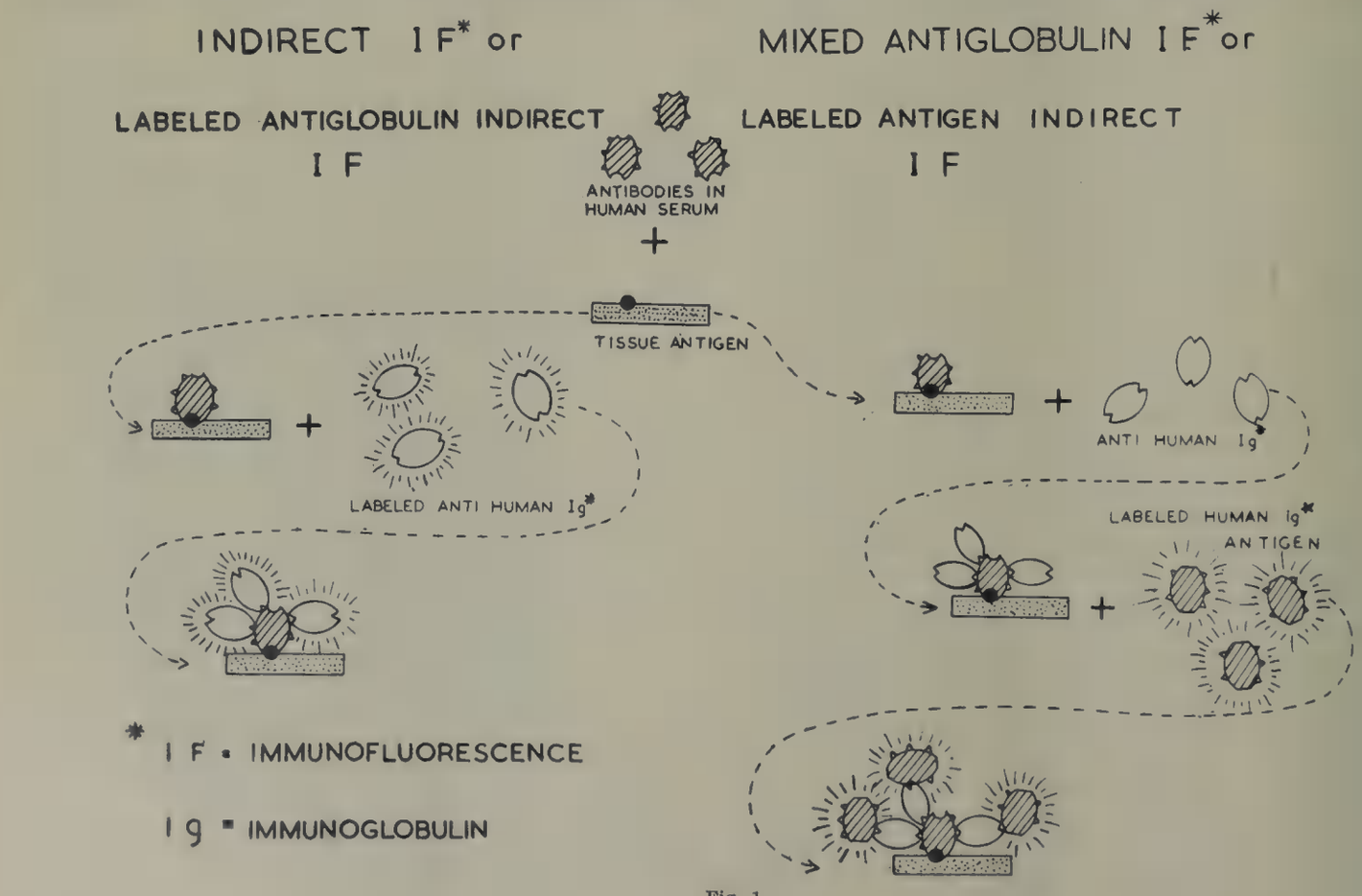

Fig. 1

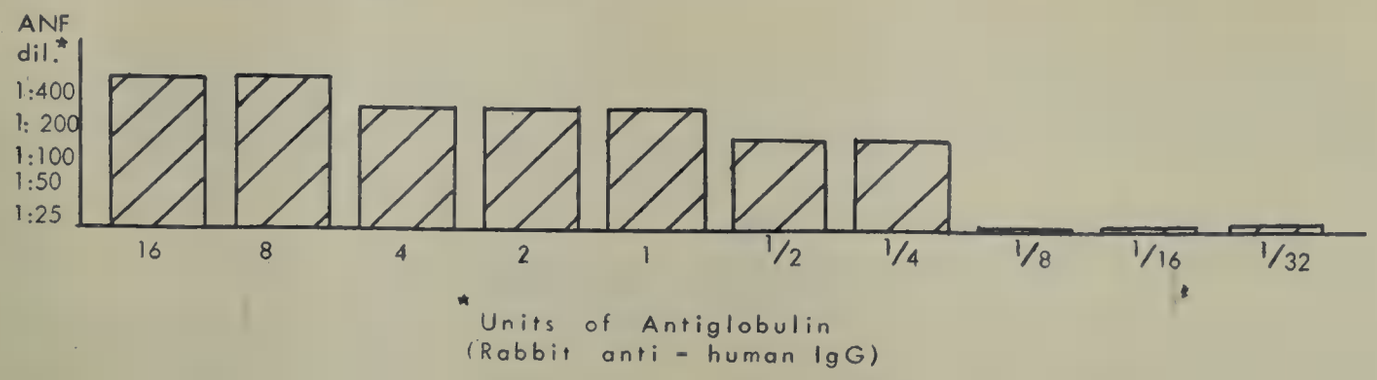

Fig. 2

yielded comparable reactions by the MIF and IIF staining methods with the rabbit antiglobulin. However, the goat antiglobulin only yielded a detectable reaction by IIF, not by the MIF staining method. This is the most discrepant result in this series of experiments; it is doubtful if much significance can be attached to it because the difference is close to the limits of error of the method (± 1 dilution).

Several types of tissue antibodies (probably all autoantibodies) were tested by both the MIF and IIF staining methods. The results of these studies are summarized in Table 3.

Table 3. Examples of Titres obtained with some Human Antibodies to Tissue Antigens by the Labelled Antigen (MIF)* and Labelled Antiglobulin (IIF)* Indirect IF* Staining Methods

Systems tested: Type of antibody	Literature ref.	Examples of titres: Tissues	By MIF	By IIF
Antinuclear factor	8, 9, 17			
Smooth		Calf thyroid	1 : 200	1 : 200
Speckled		Calf thyroid	1 : 200	1 : 400
Nucleolar		Calf thyroid	1 : 50	1 : 50
Thyroid microsomal	10	Human thyroid	1 : 100	1 : 100
Muscle striations	11, 12	Human heart	1 : 100	1 : 200
Skin antibodies:				
Intercellular	13	Guinea-pig lip	1 : 30	1 : 90
Basement zone	13	Guinea-pig lip	1 : 270†	1 : 270†
Cytoplasmic	14	Guinea-pig lip	1 : 30	1 : 30

* Abbreviations as for Table 2.
† No end-points were obtained in these particular titrations.

Antinuclear factors[8,9,17] of various sorts could be demonstrated by both MIF and IIF staining as shown in Table 3. Identical staining patterns were seen in preparations stained by the two methods. IF staining of thyroid cells[10] yielded strongly positive reactions by the labelled antigen method. Preliminary studies of muscle autoantibodies found in sera of patients with myasthenia gravis[11,12] yielded comparable reactions by the two IF staining methods. Three types of skin antibodies[13,14] could be demonstrated by MIF staining as well as IIF staining

The prime objective of these investigations on labelled antigen indirect IF staining was to seek a better understanding of the quantitative relationships that obtain in the conventional labelled antiglobulin method. The approach used in these investigations was that of chessboard titrations. Fig. 2 illustrates the results obtained in one experiment of this type. An antiglobulin monospecific for *IgG* was tested at varying dilution against doubling dilutions of an ANF. The precipitation end-point of this antiglobulin (1 unit per ml.) was 1 : 32. The labelled *IgG* antigen used for the top layer has an estimated $F : P$ ratio of 3·6/1 and was used at a concentration of 1 unit/ml. (0·05 mg protein/ml.).

The results summarized in Fig. 2 reveal that the ANF titre maintains a plateau (in the range of 1 : 200–1 : 400) down to an antiglobulin dilution of 1 : 32 (1 unit/ml.). At higher dilutions of the antiglobulin the ANF titre declines, falling off to a negative reaction at $\frac{1}{8}$ of a unit per ml.

These observations provide a basis for making the following assumptions in quantitative studies of indirect IF staining (both IIF and MIF). (1) Titration of a standard human serum yields a constant IF end-point (± 1 dilution) over a range of antiglobulin dilutions, forming a plateau in a chess-board titration as in Fig. 2. The highest antiglobulin dilution in this plateau yielding the maximal titre (± 1 dilution) of IF staining for a given human serum is the antiglobulin plateau end-point. (2) The antiglobulin plateau end-point is roughly proportional to the precipitation end-point of the antiglobulin. (3) Non-specific staining is proportional to the fluorescein concentration in the conjugate dilution used for IF staining. (4) The titre of IF staining obtained with a given human serum is proportional to the $F : P$ ratio of the labelled antiglobulin (in IIF) or labelled antigen (MIF). It must be added that over-conjugation, that is, too high a fluorescein to protein ($F : P$) ratio, so alters the protein that it gives rise to unacceptable non-specific staining[15]. (5) In labelled antigen indirect IF staining the maximal titre obtained with a given human serum is maintained over a range of dilutions of the labelled antigen of the top layer, again in a plateau fashion. The highest antigen dilution in this plateau which yields maximal titres is the labelled antigen plateau end-point. This labelled antigen end-point is also roughly proportional to its precipitation end-point.

More recent studies[12,16] have revealed that essentially these assumptions hold true for either MIF staining or IIF staining.

From the foregoing it appears that the control of the following is required for quantifying indirect immunofluorescence: (1) Characterization of antiglobulins by immunoelectrophoresis. (2) Titration of antiglobulins by precipitation tests (for MIF staining the labelled antigen must also be titrated). (3) Estimation of the fluorescein concentrations and fluorescein to protein ratios in conjugates. These parameters are minimal requirements for quantitative indirect immunofluorescence.

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EFFECT OF A LIPID FRACTION FROM RAT THYMUS ON DELAYED HYPERSENSITIVITY REACTIONS OF NEONATALLY THYMECTOMIZED RATS

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IT is now clear that the thymus has an important role in the development of immunological capacity in mammals[1]. Neonatal thymectomy is followed by a striking depletion of lymphocyte population in various lymphoid tissues[2]. The immunological role of the thymus has been considered by many investigators to be in the main indirect, this organ providing the body with cells which colonize different lymphoid tissues where they undergo further immunological maturation. Such a cellular function of the thymus does not, *a priori*, exclude the possibility that the thymus may have endocrine activity[3] and that the development of the immune system may be influenced by active principles elaborated by the thymus and discharged into the circulation[4]. Interest in this possibility has been revived recently by Szent-Györgyi *et al.*[5], who isolated two substances from thymus, among other organs, capable of affecting tumour growth. The observations presented here form part of a large experiment in which various fractions of rat thymus have been used in attempts to restore immunological potential to rats thymectomized at birth.

Thymuses from 18–28-day-old outbred albino rats were used as source for the preparation of lipid fraction: 12 g tissue was homogenized with 150 ml. of saline, followed by lyophilization of homogenate. Dried tissue was extracted with chloroform–methanol (2 : 1 by volume) at boiling-point for 12 h. The lipid fraction from whole rat central nervous tissue was prepared in a similar way. Lipid fractions were emulsified separately in saline before injection. Inbred Lewis strain rats (Microbiological Associates, Washington) were used in the experiment. Thymectomy was performed at birth, and operated animals were divided into three groups. The first group was treated with the thymus lipid fraction; the second group with the central nervous tissue lipid, and the third group was not treated. Two control groups of normal rats were also included. One control group (sham-operated) was treated with the central nervous tissue lipid, whereas another group (unoperated rats) was untreated. Each treated thymectomized or sham-operated rat received a total of 11 injections, beginning at 21 days and ending at 58 days of age. The individual inoculations contained 2·6 mg of thymus or nervous tissue lipid extract. Thymectomized animals showing the wasting syndrome were excluded from the experiment.

At 8 weeks all rats were immunized with crystallized bovine serum albumin dissolved in saline and mixed with an equal volume of complete Freund's adjuvant. 0·1 ml. of a stable water-in-oil emulsion (0·8 mg of bovine serum albumin) was injected into the left hind foot-pad. The first skin test was done 10 days, and the second 18 days, after immunization. 40 μg of bovine serum albumin in 0·1 ml. saline were injected into the skin of either left or right depilated flank. Differential leucocyte counts were performed on tail blood taken 2 days after the first skin test. At 11 weeks all animals were killed and the mediastinum was inspected grossly and histologically for the presence of thymic remnants. Axillary lymph nodes and spleen were taken for histological appraisal.

Immunological responses of thymectomized and control rats, untreated or treated with the cell-free fraction of allogeneic thymus, are given in Table 1. Delayed reactions to bovine serum albumin were sharply reduced in untreated thymectomized rats. In contrast are the delayed hypersensitive responses of thymectomized animals treated with thymic lipid. At the time of the second skin test, delayed reactivity in those rats was comparable to that of non-thymectomized controls. The responses of thymectomized rats treated with central nervous tissue lipid (CNS lipid) fell between those of untreated thymectomized rats and those of thymectomized rats repeatedly injected with thymic lipid.

Untreated thymectomized rats showed a moderate retardation in weight gain, a low incidence of wasting disease and a marked reduction in circulating lymphocytes (Table 2). The body-weight of thymectomized rats injected with thymic lipid, on the other hand, was comparable to that of sham-operated and intact controls. No wasting disease was seen in this group, and there was a partial restoration of total number of lymphocytes in the peripheral blood. Histological examination of spleen and lymph nodes from thymectomized rats, untreated or treated with central nervous tissue lipid, revealed a profound depletion of small lymphocytes, whereas the plasma cell population was found to be normal[2]. The absence of small lymphocytes was less striking in the spleen of thymectomized animals treated with thymic lipid, and in the lymph nodes masses of these cells were larger than in other thymus-free rats which were not injected with thymic lipid. There were many foci of cellular proliferation in the cortical areas of those lymph nodes. These histological findings fit fairly well with the immunological responses of thymectomized animals treated with thymus lipid fraction.

None of several thymic materials used by other authors[6] has been effective in preventing wasting disease or in

Table 2. Body-weight and Total Lymphocyte Count in Treated and Untreated Thymectomized and Control Rats

Group	No. of rats	Treatment	Average body weight at 11 weeks (g)	Average total lymphocyte count at 11 weeks
Thymectomized	10	—	170·0	6,425
	9	CNS lipid	164·1	6,591
	12	Thymus lipid	189·1	10,183
Non-thymectomized				
Intact	10	—	181·5	11,301
Sham-operated	10	CNS lipid	188·2	12,892

Table 1. Delayed Hypersensitivity to Bovine Serum Albumin in Treated and Untreated Thymectomized and Control Rats

Group	No. of rats	Treatment	Skin test at 10 days No. of rats with reaction:				Skin test at 18 days No. of rats with reaction:			
			0	+	+ +	+ + +	0	+	+ +	+ + +
Thymectomized	10	—	9	1	0	0	9	1	0	0
	9	CNS lipid	5	3	1	0	3	2	2	2
	12	Thymus lipid	3	7	2	0	1	1	4	6
Non-thymectomized										
Intact	10	—	0	2	3	5	0	2	2	6
Sham-operated	10	CNS lipid	0	1	2	7	0	3	3	4

restoring immunological competence to thymectomized mice. It has been claimed, however, that injection of thymus extracts into animals produces a temporary increase in lymphocyte number[7]. The results presented here suggest that the thymus is capable of elaborating active principles which influence immunological reactivity of thymectomized rats in terms of restoring their immune potential. One could assume that the humoral activity of the thymus, as described in this experiment, might be similar to the activity exerted by the tumour growth-promoting substance isolated by Szent-Györgyi *et al.*[5]. It should be mentioned, however, that Dalmasso *et al.*[6] failed to restore immunological potential to neonatally thymectomized animals by repeated injections of 'promine'. The reported humoral activity of the thymus seems to be related to thymic lipid, lipoprotein or lipo-soluble components. Several injections of highly polymerized deoxyribonucleic acid isolated from young rat thymus increased, to some extent, the delayed hypersensitivity response in rats thymectomized at birth (unpublished results). Thus the immunological reactivity obtained was quantitatively similar to that of rats treated with central nervous tissue lipid, but it never reached the level of delayed reactions observed in thymectomized rats injected with thymus lipid fraction.

Thymus lipid(s) exerted restorative effects on lymphocytes in the circulation and lymphoid tissues. This provides the histological substrate to the corrected immunological inadequacy, but does not itself explain the re-population of lymphoid cells, that is, whether humoral thymic factor(s) act solely by stimulating mesenchymal cells of lymphoid tissues, or through the mechanism of immune recognition. Although the present results do not offer evidence as to the thymus cells to which humoral function could be attributed, it might be assumed that this activity is related to the central cellular architecture of this organ. The implied humoral role of the thymus, however, is not inconsistent with the possibility that the thymus acts as the source of cells involved in delayed hypersensitivity responses.

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PRECIPITIN ACTIVITY IN THE SERA OF PATIENTS WITH DEFICIENCY OF β-LIPOPROTEINS (ACANTHOCYTOSIS)

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WHILE searching for isoprecipitins in the sera of multiply-transfused patients[1], the sera from two patients with a deficiency of β-lipoproteins were tested by double diffusion in agar[2] against a panel of normal human sera. Weak but definite precipitin lines formed. The lines were stained by the specific lipid-stain, sudan black *B* (ref. 3). The studies reported here were undertaken to confirm this observation and to characterize the reaction further.

Sera which had been stored at $-25°$ for approximately one year were available from the two original patients (M. S. and A. C.), and fresh specimens were obtained from two additional patients (L. Z. and N. S.) with the same disease. The precipitin reaction was detected in all of these, with the exception of a second specimen from M. S. Serum from N. S. showed the strongest reaction of the four specimens tested. The best results were obtained when reactant proportions of two (N. S. serum) to one (normal serum) were used. Precipitin lines that developed within 16 h and stained with sudan black *B* were observed between N. S. and 29 of 30 sera from American white individuals. Under similar conditions sera from L. Z. and A. C. gave weaker and less-well-defined reactions. The low-density lipoproteins (specific gravity $< 1{\cdot}063$) from 36 individuals were isolated by flotation in a salt medium of specific gravity 1·063 by the method of Havel *et al.*[4]. Thirty-five of these 36 isolated lipoprotein specimens reacted with serum from L. Z., A. C. and N. S. (Fig. 1). The isolated lipoproteins failed to react with any of ten-fold concentrated sera from ten normal, white individuals. The $> 1{\cdot}063$ fraction of the fractionated sera did not produce a precipitin reaction even when concentrated by ultrafiltration to a degree comparable to that of the low-density lipoprotein fraction (ten-fold concentrated).

In order to characterize the precipitin in the sera of the acanthocytosis patients, the immunoelectrophoretic method of Grabar and Williams[5] was modified as follows. Sera from A. C. and L. Z. were concentrated three-fold by ultrafiltration, placed in the centre well, and subjected to electrophoresis in 1 per cent agar in veronal buffer, *p*H 8·6, ionic strength 0·05, for 1 h at 6 V/cm. Isolated low-density β-lipoprotein was placed in one trough and whole serum in the other. A single arc developed when the whole serum was used, and two arcs formed opposite the isolated low-density β-lipoprotein. The arc common to both troughs was broad, with indistinct borders. It had a cathodal mobility intermediate to that of β- and γ-globulins. The second arc had an anodal migration which corresponded to that of albumin or an α_1-globulin, and was about one-half as intense as the slower arc. Both arcs tended to form very near the trough, and neither alteration of the concentration of reactants nor moving the trough farther from the well influenced their position significantly (Fig. 1). The clearest arcs were noted when isolated lipoprotein diluted 1 : 3 with saline was used.

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Specific goat anti-human immunoglobulin antisera obtained from Dr. W. Terry, National Institutes of Health, were used in an attempt to relate the precipitin activity in the acanthocytosis serum to that of three of the known immunoglobulins (γA, γM and γG)[6]. The position of the major component was similar to but not identical with that of the γA-immunoglobulin (Fig. 2). Serum from patient A. C. was absorbed in the test-tube and in agar gel with these three antisera, but the precipitin activity was not removed. Five ml. serum from patient A. C. was subjected to column chromatography on DEAE in phosphate buffer at *p*H 7·0. The serum was placed on a column 1 cm × 25 cm and the protein fractions were eluted in a stepwise fashion using 0·005, 0·025 and 0·5 M phosphate buffers. The precipitin activity could not be demonstrated in any of the four fractions obtained despite five- to ten-fold concentration of each of the fractions.

Serum from patient A. C. was tested, using the Ouchterlony technique, against the isolated β-lipoprotein specimens from 24 normal individuals. This same panel was tested with the human anti-β-lipoprotein antiserum C. de B.[7]. Nineteen of the 24 specimens reacted with C. de B. while all 24 of the specimens reacted with serum from patient A. C. The precipitin reaction with A. C. occurred after 4 h, while that with C. de B. occurred after 16 h. Precipitin arcs with C. de B. formed approximately midway between the centre (antiserum) and peripheral (panel sera) wells, while that with patient A. C. formed immediately adjacent to the peripheral well. These observations suggest that the precipitins in the acanthocytosis sera are different from those developed in multiply-transfused patients. Other experiments were performed which showed that the precipitin band stains only weakly, if at all, with the protein stains, azo carmine and amido black 10*B*, but strongly with lipid stains. Repeated testing of a single normal serum specimen under the same experimental conditions usually, but not always, produced a precipitin line. The failure of precipitin band formation was not related to variations in *p*H, temperature, or buffer composition. The reaction did not fix complement using the technique of Sever[8]. The complex formed between the C. de B. antiserum and β-lipoprotein does not fix complement, but the reaction does conform to most other criteria for an antigen–antibody reaction[9].

These findings and several recent reports in the literature suggest that the reaction observed is probably a non-specific precipitation reaction.

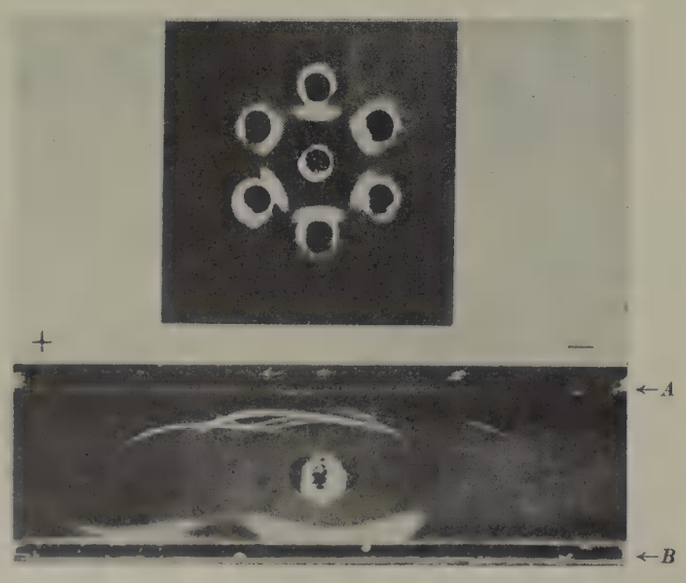

Fig. 1. The upper portion shows a representative Ouchterlony pattern. The centre well contains serum from a patient with deficiency of β-lipoproteins. Peripheral wells contain isolated low-density β-lipoproteins. The lower portion is an immunoelectrophoretic pattern of the serum from an acanthocytotic patient showing precipitin activity in the β_2-γ-globulin and α_1-globulin regions. Trough *A* contains horse anti-human antiserum. Trough *B* contains isolated low-density β-lipoproteins

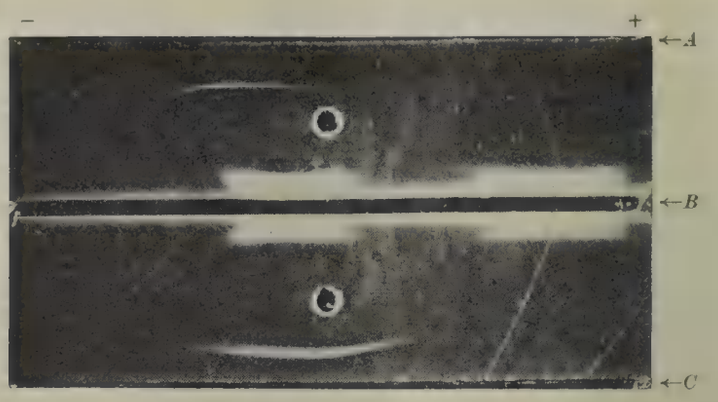

Fig. 2. Immunoelectrophoretic pattern of the serum of a patient with acanthocytosis, comparing the position of the precipitin region with that of known immunoglobulins. Wells contain the serum of patient A. C. Trough *A* contains goat anti-γM antiserum. Trough *B* contains isolated low-density β-lipoproteins. Trough *C* contains goat anti-γA antiserum

Tomasi[10] noted that certain extracts of human organs gave a non-specific precipitation reaction with normal human sera using a modified Ouchterlony technique[2]. He presented evidence that albumin was the reactive component in the serum and that the tissue extract component migrated slightly toward the cathode on immunoelectrophoresis. Teichman and Vogt[11] also noted non-specific precipitation reactions occurring between the sera of patients with tuberculosis and old tuberculin obtained from an *H37Rv* strain of *M. tuberculosis*. They identified the serum component as an α_1-lipoprotein by its immunoelectrophoretic mobility. The same serum component was also found in sera of normal individuals as well as in sera of patients with carcinoma of the lung. They further suggest that Tomasi's serum component as well as that of Peetom *et al.*[12] (who reported a precipitation reaction between normal human and certain animal sera and haemolysates of normal human red cells) are also α_1-lipoprotein in nature. Other authors[13,14] have reported non-specific precipitin reactions between tissue extracts and normal sera, but such a reaction has not been previously reported between two human sera or with the low-density β-lipoprotein of human sera. In addition, precipitin activity is found in two fractions of the acanthocytotic serum. It is possible, however, that the α_1-globulin fraction contains antibody activity, since G. Strejan and I. Flechner were able to produce antibody with α_1-globulin mobility in rabbits[15].

In summary, the following findings support the hypothesis that the precipitin activity in the acanthocytosis patients is probably non-specific.

(1) The precipitin arcs on immunoelectrophoresis have an atypical appearance compared with those seen using human, anti-human lipoprotein antisera such as C. de B.

(2) Precipitin activity is noted in the albumin or α_1-globulin region. Non-specific precipitin reactions, which have been previously described, also occurred in these regions.

(3) The precipitin activity was not removed by absorption with three common anti-immunoglobulin antisera.

(4) Precipitin activity has been demonstrated with nearly all the normal sera against which it has been tested.

(5) Precipitin bands stain well with lipid stains but weakly, if at all, with protein stains.

(6) It is unusual for antibody activity against the same antigen to develop in two classes of immunoglobulins in the same individual.

(7) The complex formed does not fix complement.

Neither demonstration of a classical antigen–antibody precipitation curve nor localization of the precipitin activity in the β- or γ-globulin region on immunoelectrophoresis is sufficient evidence to classify the reactant in the acanthocytosis patient's serum as an antibody[14]. Nieco and Barrett[13] noted an atypical precipitin band when chymotrypsin was tested in agar against an anti-

carboxypeptidase antiserum produced in rabbits. The band was unlike that produced with carboxypeptidase *A* and stained with sudan black but not with bromphenol blue. The antiserum would neither agglutinate latex particles coated with chymotrypsin nor provoke passive cutaneous anaphylactic reactions in guinea-pigs with chymotrypsin as antigen. Both phenomena occurred when carboxypeptidase *A* was used as antigen. They concluded that the precipitin was spurious, probably resulting from precipitation in the agar of lipid released by enzymatic degradation of the lipoproteins in the antiserum. The precipitin bands noted in our experiments are similar to theirs and may also result from the action of an enzyme or enzymes in the serum of these patients with deficiency of β-lipoproteins.

A reaction between the low-density β-lipoproteins and two components in the serum of patients with deficiency of β-lipoproteins is clearly demonstrated for the first time. Further work is required to determine whether these components are peculiar to the sera of acanthocytotic patients and whether they are a result of, or play a part in production of, the deficiency of β-lipoprotein.

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DOUBLE CHROMOSOME REDUCTION IN A TETRAPLOID *SOLANUM*

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REPEATED attempts to cross *Solanum demissum* ($2n = 6x = 72$) with *S. stoloniferum* ($2n = 4x = 48$) gave one seeded berry containing one seed. This yielded a plant (*M*.271) which had $2n = 4x = 48$, instead of the expected $2n = 5x = 60$. The cytological, morphological and biochemical studies of *M*.271 reported here provide good evidence that it is a true hybrid and therefore the product of double chromosome reduction.

The plants used in this investigation were as follows: *Parents*. *Solanum demissum* (C.P.C. 2249) (*dms* 2249). This clone, collected by Dr. J. G. Hawkes in 1949 in Hidalgo State, Real del Monte, Mexico, is morphologically typical of the species. It is both male and female fertile, producing 94·6 (± 1·64) per cent stainable pollen of a regular diameter and a mean of 96 seeds per berry on self-pollination. *Solanum stoloniferum* (C.P.C. 2282.2) (*sto* 2282.2). This clone was grown from seed collected by Dr. J. G. Hawkes in 1949 in Puebla State, Mexico. It is a form of the species characterized by dark green leaves, many interjected leaflets, a high leaflet index and blue flowers. It sets abundantly seeded berries by self-pollination.

Hybrid M.271. This one seed obtained in the cross *dms* 2249 (♀) × *sto* 2282.2 (♂) germinated and grew very slowly into a small plant with extremely short internodes and a tufted, almost cushiony habit; it produced only a single flower. By contrast, when grafted on tomato, its growth was upright and vigorous, it produced long internodes and flowering was profuse and continuous. Comparison of the leaf and flower characters of *M*.271 with those of its parents (Table 1) shows that in most ways it resembles the *demissum* parent more closely than it does *sto* 2282.2. A predominance of *demissum* would also be expected in a pentaploid hybrid ($2n = 60$—3 *dms* + 2 *sto* genomes). Morphologically, *M*.271 resembles polyhaploid *S. demissum* ($2n = 3x = 36$)[1-3] and could be taken for one were it not for its chromosome number. An analysis of meiosis gave the results shown in Table 2.

Table 1. Leaf Characters of Parents and Hybrid

	dms 2249 Mean* (range)	*M*. 271 Mean* (range)	*sto* 2282.2 Mean* (range)
Pairs of lateral leaflets	2·90 (2–3)	2·00 (0)	3·40 (3–4)
Number of interjected leaflets	2·55 (1–4)	0·90 (0–1)	6·40 (3–10)
Terminal leaflet index $\left(\frac{\text{Breadth}}{\text{Length}}\%\right)$	58 (52–68)	61 (50–72)	66 (60–73)

* Based on ten measurements.

M.271 has no stainable pollen and attempts to backcross it by both parents as males gave one berry when *sto* 2282.2 was used. This contained two seeds, but neither germinated.

Possible Origins

There is no definite morphological evidence to suggest, let alone prove, that *M*.271 is a true hybrid of *S. demissum* × *S. stoloniferum*. Before giving the biochemical evidence on this point, we may consider three other possible origins, as follows:

(1) *Contamination*. It is possible that *M*.271 is a hybrid between *dms* 2249 and a diploid *Solanum*, since three such species, *S. chomatophyllum*, *S. santolallae* and *S. verrucosum*, were growing in the same glasshouse as *S. demissum* and were being used in the same crossing programme. However, all attempts to cross *demissum* with these diploids failed, and, in addition, *M*.271 shows no evidence of the characteristic morphology or phenolic pattern (see later) of any of these species. Hybrids between *demissum* and *verrucosum* have been recorded[4,5]: they differ both morphologically and cytologically from *M*.271.

Table 2. Meiotic and Fertility Data

Plant	Meiosis in pollen mother cells							Per cent ± *S.E.* stainable pollen
	No. of cells	Mean ± *S.E.* per cell					Term. coeff.	
		IV	III	II	I	Xta		
sto 2282.2	25	0	0	24·00	0	32·64 ± 0·51	0·56	60·4 ± 4·02
M.271	25	0·08	1·48 ± 0·25	12·64 ± 0·36	17·96 ± 0·40	22·4 ± 0·73	0·72	0

(2) *Female parthenogenesis.* Polyhaploid plants of *S. demissum* are occasionally produced as a result of female parthenogenesis in selfed progenies of *S. demissum*[3] or in progenies obtained from crosses between *demissum* and diploid species[2,6,7]. All had $2n = 3x = 36$ except for two plants: one which Dodds[2] found to have $2n = 3x + 3 = 39$ and another which Beamish[7] found to have $2n = 2x + 2 = 26$.

A tetraploid plant could arise parthenogenetically from a hexaploid only by an extremely abnormal maternal meiosis. Meiosis and embryo-sac development were therefore studied in ovules of *dms* 2249, using sections stained with Heidenhain's haematoxylin. Meiosis was regular and embryo-sac development was of the type normal for *S. demissum*[8]. Thus the occurrence of a parthenogenetic tetraploid seedling of *demissum* is extremely unlikely.

(3) *Male parthenogenesis.* *M.*271 could have conceivably arisen by male parthenogenesis, in which case it would be genetically equivalent to a segregate in a progeny raised by selfing *sto* 2282.2. Such a progeny was grown and all the individuals were found to resemble closely the parent plant; furthermore, all were fertile and set berries by self-pollination. Thus, this parental clone is highly homozygous, and it is therefore inconceivable that a male parthenogenetic seedling should resemble *demissum* rather than its *stoloniferum* parent.

Biochemical Evidence

Except for the anthocyanin pigments of flowers which exhibit dominant-recessive relationships in their inheritance, the genetic control of most chemical constituents is more or less quantitative and hybrids contain most or all of the substances present in the parents[9]. Thus chemical constituents characteristic of presumed parents can give good evidence of the origin of a putative hybrid. On this assumption, the flavonol glycosides of the flowers and the leaf alkaloids of *dms*, *sto* and *M.*271 were examined, since these substances were known to show significant variation among the wild tuber-bearing *Solanum* species.

Phenolic patterns in flowers. Ten flavonol glycosides are known to occur (and vary) in potato flowers[10]. Their distributions in *M.*271, in its putative parents and in three related diploid species were therefore examined (Table 3). The results eliminate the possibility that *M.*271 could have arisen by a chance hybridization between *demissum* and a related diploid species. The following points about the phenolic patterns of the flowers may be noted.

(1) The pattern of *M.*271 is identical with that of *demissum*, agreeing with the morphological evidence (see earlier) showing that *demissum* is the predominant parent.

(2) The pattern of *M.*271 does not disagree with the idea that *stoloniferum* is the other parent. One constituent of *stoloniferum*, it is true, is not present in the hybrid. This substance, luteolin 7-glucoside, though characteristic of the species, is not always present: it was lacking in four out of 38 clones examined[10]. Its absence from *M.*271 is therefore not critical.

Table 3. Phenolic Constituents of Flowers

	Occurrence in					
	dms 2249	*M.* 271	*sto* 2282.2	*chm** 2708	*san* 2519	*ver* 2644
Flavonol glycosides						
Kaempferol 3-glucoside	−	−	−	−	+	−
Kaempferol 3-rutinoside	−	−	−	−	−	+
Kaempferol 3-sophoroside	−	−	−	+	+	−
Kaempfero 3-(2^G-glucosylrutinoside)	−	−	−	+	−	+
Quercetin 3-glucoside	−	−	−	−	+	−
Quercetin 3-rutinoside	+	+	+	−	−	+
Quercetin 3-sophoroside	−	−	−	−	+	−
Quercetin 3-(2^G-glucosylrutinoside)	−	−	−	+	−	+
Myricetin 3-rutinoside	+	+	−	+	−	−
Luteolin 7-glucoside	−	−	+	−	−	−
Other phenolics						
Caffeic-glucose-quinic complex	+	+	−	−	−	+
Unknown colourless→mauve	−	−	−	+	−	−

* Also contains two other unidentified flavonol glycosides.
Key: *dms—demissum; sto—stoloniferum; chm—chomatophyllum; san—santolallae; ver—verrucosum.*

Table 4. Alkaloids in the Leaves

Plant	Alkaloid	$R^*_{\alpha\text{-}S}$	R_F†	Products of acid hydrolysis: Aglycone(s)	Sugars
dms 2249	Demissine	0·94	0·29	Demissidine	Xylose, glucose and galactose
sto 2282.2	Solanine α-chaconine	1·0 1·35	0·28 0·46	Solanidine	Rhamnose, glucose and galactose
*M.*271	Demissine (?) New demissidine glycoside α-chaconine (?)	0·88 ‡ 1·37	0·30 0·48	Demissidine + traces of solanidine§	Rhamnose, xylose, glucose and galactose

* R_F relative to α-solanine: chromatograms run on citrate-buffered Whatman No. 1 paper in butanol–citric acid–water.
† Chromatograms run on silica-gel plates in acetic acid–ethanol (1 : 3).
‡ Identified as a mixture of two components, since this spot gave a strong Dragendorf reaction for alkaloid but only a weak reaction with Clarke's reagent (used for detecting solanidine glycosides in the presence of demissidine glycosides, which do not react).
§ Solanidine was detected, in the presence of demissidine, by the use of Sarett's reagent (compare ref. 13). The crude mixture of alkaloid aglycones failed to respond to the nitroso test, indicating that tomadine was absent.

(3) The pattern of *M.*271 is quite different from those of the three diploid species (Table 2), which makes it very unlikely that any of these species is involved in its parentage. Thus, all three not only contain kaempferol as well as quercetin glycosides but, in addition, the glycosidic patterns (sophoroside, glucoside and (2^G-glucosylrutinoside)) are quite different from *M.*271 or its parents (which have only the 3-rutinoside). Production of (2^G-glucosylrutinoside) in flowers of *S. stoloniferum* and *S. chacoense* is known[10] to be controlled by a dominant gene *Gl* (3-rutinoside being the recessive character), and it is unlikely that this glycosidic form would not appear in hybrids involving *chomatophyllum* or *verrucosum*.

Alkaloids of the leaves. Steroidal alkaloids occur, combined with sugars (branched tri- or tetra-saccharides), quite characteristically in the genus *Solanum*, and their distribution has been studied intensively in recent years[11]. While glycosides of solanidine are widely distributed in the tuberous solanums, those based on demissidine are found only in *S. demissum*. Thus it should be possible to characterize hybrids of *demissum* with other species on the basis of their alkaloid content and this has, in fact, been done with *M.*271.

The alkaloids of both parents were examined, with the results shown in Table 4. The clone of *S. demissum* contained the expected demissine[12] and *S. stoloniferum* contained solanine and α-chaconine, the two commonest potato alkaloids. *M.*271 contained three glycosides of which two corresponded in R_F value with glycosides from the different parents, that is with demissine and α-chaconine; the third appeared to be a new demissidine glycoside. On acid hydrolysis, these glycosides together yielded demissidine, with traces of solanidine, and four sugars: glucose, galactose, xylose and rhamnose. Thus, *M.*271 has the aglycones of both parents and also the constituent sugars of both, since the bound xylose is derived from *S. demissum* and the bound rhamnose can only have come from *S. stoloniferum*.

Since major intraspecific variation in alkaloid content is unknown in the potatoes, this result is effective proof that *M.*271 is a true hybrid between *S. demissum* and *S. stoloniferum*.

Origin of M.271

The morphology and biochemistry of *M.*271 indicate that it is far nearer its female parent, *demissum*, than it is to *stoloniferum*, suggesting that double chromosome reduction occurred in the latter, the male parent.

Meiosis was normal in the sample of pollen mother cells examined from *sto* 2282.2 (Table 1). However, pollen grain measurements from ten different anthers showed a range of size within each anther. Using within-anther variance as a measure of this variation, Bartlett's test showed a highly significant heterogeneity between anthers ($\chi^2_{[9]}$ 487.4). Furthermore, the mean variance within anthers of *sto* 2282.2 was above that for other *stoloniferum* clones. Pollen stainability was also extremely variable in *sto* 2282.2, ranging from 12 to 75 per cent per anther. Of a

sample of 1,000 'good' pollen grains 5·6 per cent had diameters equal to or below the modal diameter of pollen grains measured in a diploid species. Since, within species, the size of a pollen grain is often correlated with its chromosome number, it is quite likely that some of the small pollen grains found in *sto* 2282.2 were double-reduced, with 12 chromosomes.

Matsubayashi[14] found that certain clones of *stoloniferum* produced occasional tripolar spindles at *M*I of meiosis which resulted in hexads containing six nuclei of almost equal size. Such a mechanism could well be the source of double-reduced pollen grains. Unfortunately a re-investigation of meiosis in *sto* 2282.2 is impossible since the clone was lost through virus infection. There is therefore no information about the cytological mechanism of the presumed double reduction.

Discussion

Double reduction has been invoked on a number of occasions to account for the appearance of unusual hybrids. Thus Steere[15] obtained diploid progeny which resembled both parents, from the cross *Petunia axillaris* ($2n = 2x = 14$) × *P. hybrida* ($2n = 4x = 28$). Nishiyama[16] made similar observations on *Avena* hybrids, Karprechenko[17] on *Brassica* × *Raphanus* crosses and Ewart and Walker[18] on *Poinsettia*. In the cherry, three diploid cultivars are reputed to have arisen from crosses between diploid *Prunus avium* and a tetraploid cultivar, 'May Duke'[19]; Darlington[20] thought that the chromosome number of the parents disproved the supposed origin of these cultivars but, in retrospect, it now seems that the original interpretation is worth reconsideration.

In none of these examples is there definite proof of double chromosome reduction: for the potato hybrid, although direct cytological evidence is lacking, the biochemical data appear to be decisive. The fact that Matsubayashi[14] independently obtained a tetraploid plant from the same cross that produced *M*.271 suggests that double chromosome reduction may be less uncommon than we think. But it seems unlikely that it is an important general mechanism in Nature for decreasing chromosome numbers.

From a biochemical point of view, *M*.271 is particularly interesting since it contains a new hybrid substance, a glycoside of demissidine not present in either parent. The identification of the sugar linkages in this glycoside will clearly be of interest, as it should throw some light on the control of alkaloid glycoside synthesis, about which very little is at present known.

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RETICULOCYTE RIBOSOME FRACTION WITH AN EXCEPTIONAL CAPACITY FOR POLYPHENYLALANINE SYNTHESIS

By Dr. JOHN O. BISHOP
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RETICULOCYTE ribosomes, isolated from the cell lysate by differential centrifugation, form a heterogeneous population with respect to size[1–4]. The accepted unit is the ribosomal monomer, with a sedimentation coefficient in the region of 80 *S* (ref. 5). Polyribosomes[4] (or ergosomes[6]) consist of aggregates of two or more monomers, held together probably by a strand of informational RNA. Two types of ribosomal sub-units are also found, with sedimentation coefficients of about 60 *S* and 40 *S* (ref. 3).

It is widely recognized that the monomer is the active unit in protein synthesis. However, the monomers found free in the cell lysate do not synthesize protein[2–4]; those ribosomal monomers which make up the polyribosome do so, each monomer synthesizing a single polypeptide chain[7,8]. In this communication the term 'monosome'[9] is used to refer to any ribosomal monomer which is not incorporated in a polyribosome. It is convenient to be able to refer in molar terms to the monomers which constitute the polyribosomes, and to the monomers which would be formed by the aggregation of a given quantity of sub-units. The term 'monomer equivalent'[7] is used to refer to the amount of any given homogeneous or heterogeneous ribosome preparation equal in mass to a ribosomal monomer. The term 'native', used to describe a particular ribosome species, indicates that it was found in the cell lysate. This does not imply that it is present as such within the cell. A 'derived monosome'[10] is one released from a polyribosome during protein synthesis in the cell-free system. 'Derived sub-units' are sub-units prepared by dissociation of monosomes[5].

The macromolecular components required for the synthesis of polypeptide under the direction of synthetic polynucleotides are activating and transfer enzymes, transfer RNA, and ribosomes. In the preparations used, the predominant ribosomal species has usually been the monosome. The newly synthesized polypeptide chains are sometimes found associated with artificial polyribosomes which are thought to consist of two or more ribosomal monomers attached to a single polynucleotide molecule[11–14]. These sediment in a centrifugal field more rapidly than the monosomes[11–16]. In none of the cases cited has more than a small proportion of the available ribosomal material been seen to become involved in the formation of complexes with polynucleotide. This suggests that only a minor ribosome fraction is capable of reacting with the synthetic polynucleotide.

This conclusion has recently been confirmed by measuring the chain-length of the polyphenylalanine which is synthesized in the reticulocyte cell-free system under the direction of polyuridylic acid (poly U)[17]. Total phenylalanine incorporation in this system rarely exceeds 5 mμ moles per mg of ribosomes, or, taking the molecular weight of the monomer to be 4×10^6 (ref. 21), 20 phenylalanine residues per monomer equivalent. The average chain-length was found to be of the order of 200 phenylalanine

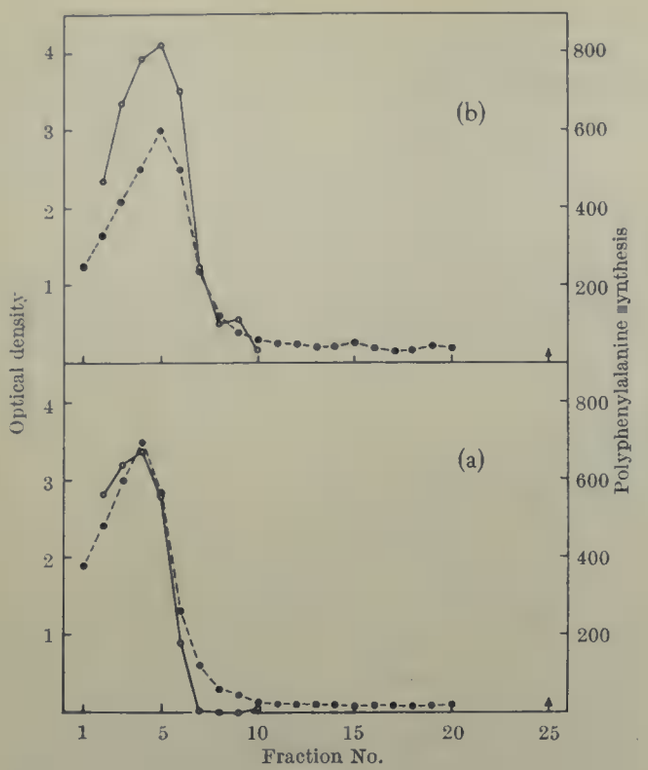

Fig. 1. Polyphenylalanine synthesis by native and derived monosomes following zone sedimentation. (*a*) Native monosomes. (*b*) Derived monosomes. The broken lines show optical density at 260 mμ: solid lines show polyphenylalanine synthesis, measured as c.p.m. incorporated per 0·2 ml. under the standard assay conditions described below. The direction of sedimentation is from right to left. The preparation of monosomes is described in the text. One ml. of the monosome fraction, in standard buffer containing 5 per cent sucrose, was layered on a 29 ml., 10–23 per cent sucrose gradient, prepared in standard buffer, and centrifuged for 10 h at 25,000 r.p.m. in the Spinco 'No. 25' rotor at 0° C. Suitable dilutions of fractions 1 to 10 were assayed spectrophotometrically. Fractions 2 to 10 were assayed for polyphenylalanine synthesis in the cell-free system by mixing 0·2 ml. with an equal volume of a solution containing, in μmoles: ATP, 0·28; GTP, 0·08; creatine phosphate, 2·8; $MgCl_2$, 2; *tris*-HCl, *p*H 7·2, 16; glutathione, 6; 17 L-amino-acids, 0·01 each; and uniformly labelled ^{14}C-phenylalanine, specific activity 10 c./mole (approximately 5,000 c.p.m. per μmole), 0·02; together with 5 μg creatine kinase, 50 μg yeast *s*RNA[19], 25 μg poly-U and 1·5 mg of an enzyme fraction prepared by precipitating the ribosomal supernatant between 40 and 70 per cent ammonium sulphate saturation[7]. The optimum amount of KCl (20 μmoles) was introduced with the gradient fraction. The mixtures were incubated for 40 min at 37° C and chilled. 0·1 ml. of 6 N NaOH was added, and after 2 min at 37° C the mixtures were again chilled and 2 ml. of 10 per cent TCA was added. After 30 min at room temperature the precipitates were collected on 2-cm diameter 'Oxoid' membrane filters, washed 8 times with 5 per cent TCA, twice with ethanol/ether (1/1) and once with ether. Radioactivity was measured using a 'Tricarb' liquid scintillation counter, by placing the filter in a 20 ml. vial containing 15 ml. of counting fluid (0·5 per cent w/v PPO, 0·03 per cent w/v dimethyl POPOP in toluene)

residues[17]. If one monomer equivalent is involved in the synthesis of each chain, then only 10 per cent of the ribosomal material is active in polyphenylalanine synthesis.

This paper shows that two minor ribosomal species, the native ribosomal sub-units, are very active in polyphenylalanine synthesis. Both types of sub-units are required, together with poly-U. The specific activity of sub-units is 3–4 times greater than that of monosomes. Up to 80 phenylalanine residues are incorporated in the cell-free system per sub-unit pair.

The ribosomes used in these experiments were prepared from rabbit reticulocytes[18]. The cells were washed 3 times by centrifugation and lysed by stirring for 2 min at 0° C with 3 volumes of buffer (10 mM *tris*-HCl, *p*H 8·1 at 0°, 30 mM KCl, 2 mM $MgCl_2$). Lysis was terminated by adding 0·45 times the original cell volume of ice-cold 2 M sucrose. Cell debris was removed by centrifuging for 15 min at 13,000*g*. Ribosome fraction *P*1 was the pellet obtained by centrifuging the clarified lysate for 1 h at 30,000 r.p.m. in the Spinco 'No. 30' rotor (78,000*g*). Measurements[7] made with the analytical ultracentrifuge with Schlieren optics showed *P*1 to contain polyribosomes and monosomes in the approximate proportion of 2 : 3, together with a trace (about 5 per cent) of ribosomal sub-units. Fraction *P2H* was the pellet obtained by centrifuging the lysate for 2 h. The composition of

Table 1. Endogenous and Poly-U-stimulated Phenylalanine Incorporation by Ribosome Fractions

Conditions	Fraction *P2H*	Fraction *P*2–4
Complete system	0·644	0·219
Plus 40 μg poly-U	2·895	2·040

One mg of the ribosome fraction was incubated at 37° C for 40 min in a total volume of 0·4 ml. containing other reagents in the amounts given in the legend to Fig. 1. The samples were precipitated, collected, washed and counted as described there. Phenylalanine incorporation is presented as mμ moles of phenylalanine incorporated per mg of ribosomes.

P2H was similar to that of *P*1, but it contained higher proportions of monosomes and sub-units. Fraction *P*2–4 was the pellet obtained by centrifuging the 2-h supernatant for 4 h. This fraction consisted largely of monosomes (about 70 per cent) and sub-units (20 per cent), and contained no polyribosomes except for a small proportion of dimers (about 10 per cent). Purified native monosomes were prepared by zone centrifugation: 1 ml. (15–20 mg) of fraction *P*1 in standard buffer (0·1 M KCl, 1 mM *tris*-HCl, *p*H 8·1 at 0° C, 1·5 mM $MgCl_2$) containing 5 per cent sucrose was layered on a 29-ml., 10–23 per cent sucrose gradient prepared in standard buffer, and centrifuged for 8 h at 25,000 r.p.m. (Spinco 'No. 25' rotor). The bottom 5 ml. of the gradient was diluted with 15 ml. of standard buffer and centrifuged for 3 h at 78,000*g* to obtain a pellet of purified native monosomes. The pellet from the 8-h gradient contained polyribosomes virtually free of monosomes. These were gently resuspended and 'derived' monosomes were prepared from them by incubation in the complete cell-free system (see legend to Fig. 1). The mixture was diluted with 7 volumes of standard buffer, and a pellet, which contained both derived monosomes and residual polyribosomes, was obtained by centrifuging for 3 h at 78,000*g*. The derived monosomes were separated from polyribosomes during the subsequent analysis (Fig. 1). 40-*S* native ribosomal sub-units were purified by centrifuging 1 ml. of fraction *P*2–4 through a 29-ml. 10–23 per cent sucrose gradient (in standard buffer) for 10 h at 25,000 r.p.m. in the Spinco 'No. 25' rotor. 40-*S* sub-units were well resolved, but 60-*S* sub-units appeared

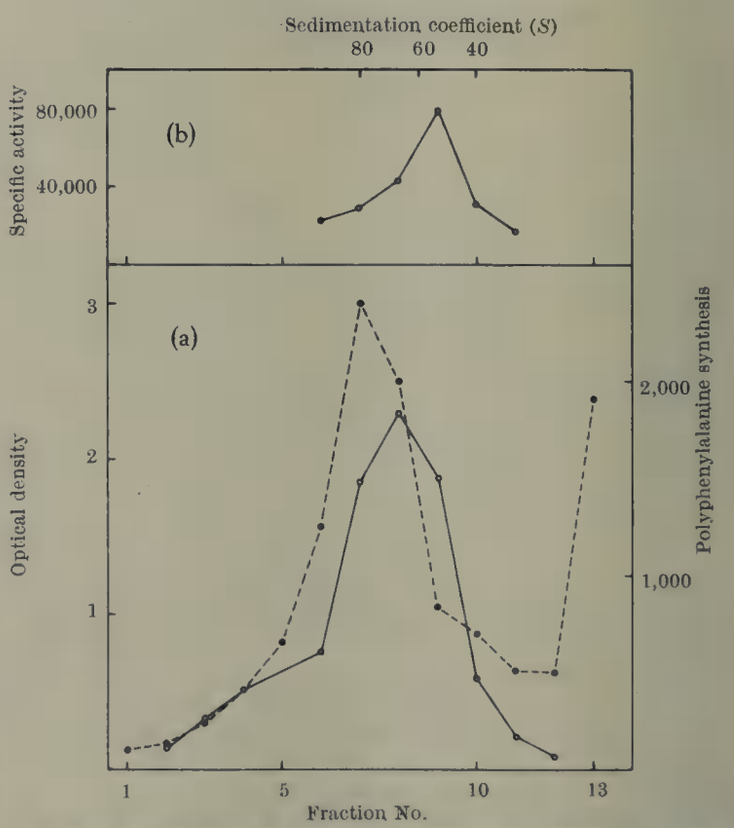

Fig. 2. Polyphenylalanine synthesis by ribosomal fractions following zone sedimentation. (*a*) Optical density at 260 mμ (broken line) and polyphenylalanine synthesis (solid line) measured as c.p.m. incorporated under the standard assay conditions described in the legend to Fig. 1. (*b*) Specific activity, as c.p.m. incorporated per mg of ribosomal material. Direction of sedimentation from right to left. Fraction *P*2–4 (1 ml.) was applied to a 29-ml. sucrose gradient (10–23 per cent) in standard buffer and centrifuged at 25,000 r.p.m. for 5 h. Thirteen 2·2-ml. fractions were collected and assayed as described in the legend to Fig. 1

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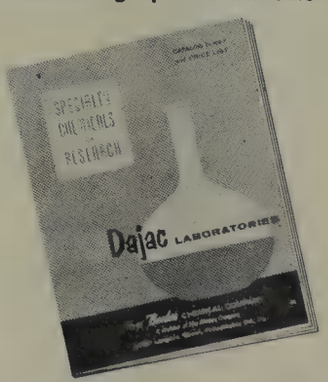

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(1) E. Gurr (1965), Rational Use of Dyes in Biology & General Staining Methods. (Leonard Hill (Books), London. Williams & Wilkins, Baltimore.)

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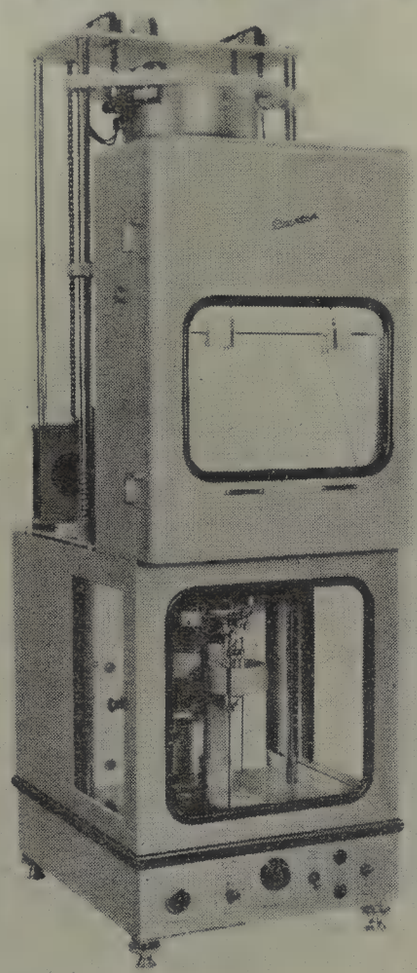

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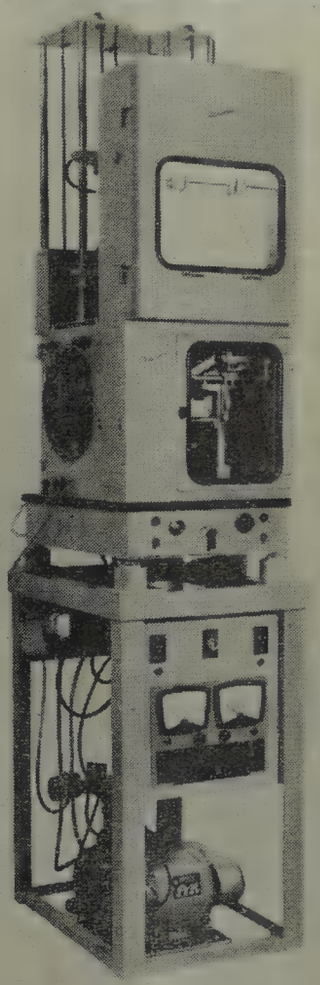

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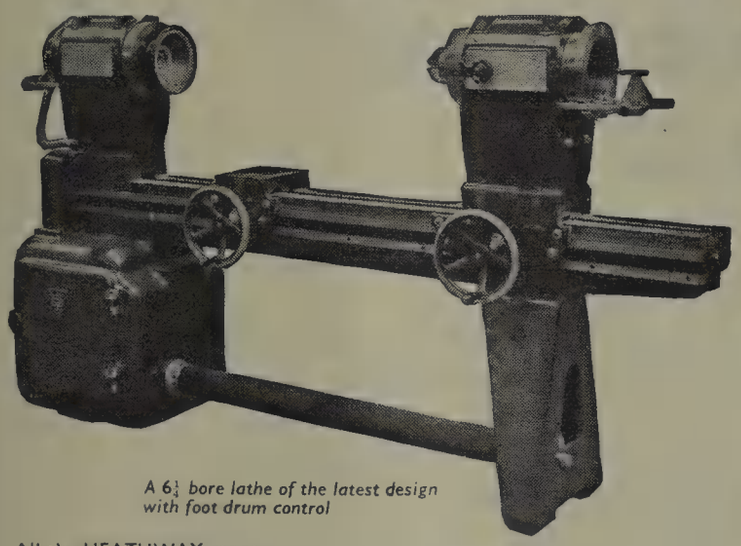

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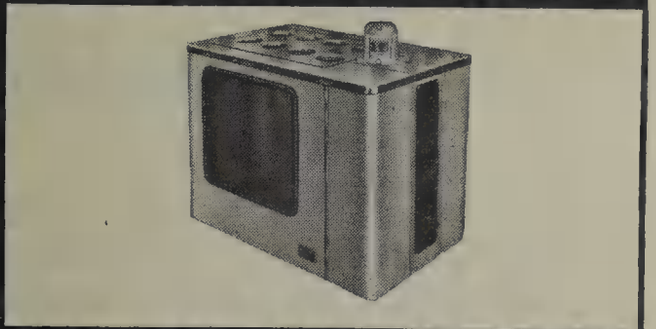

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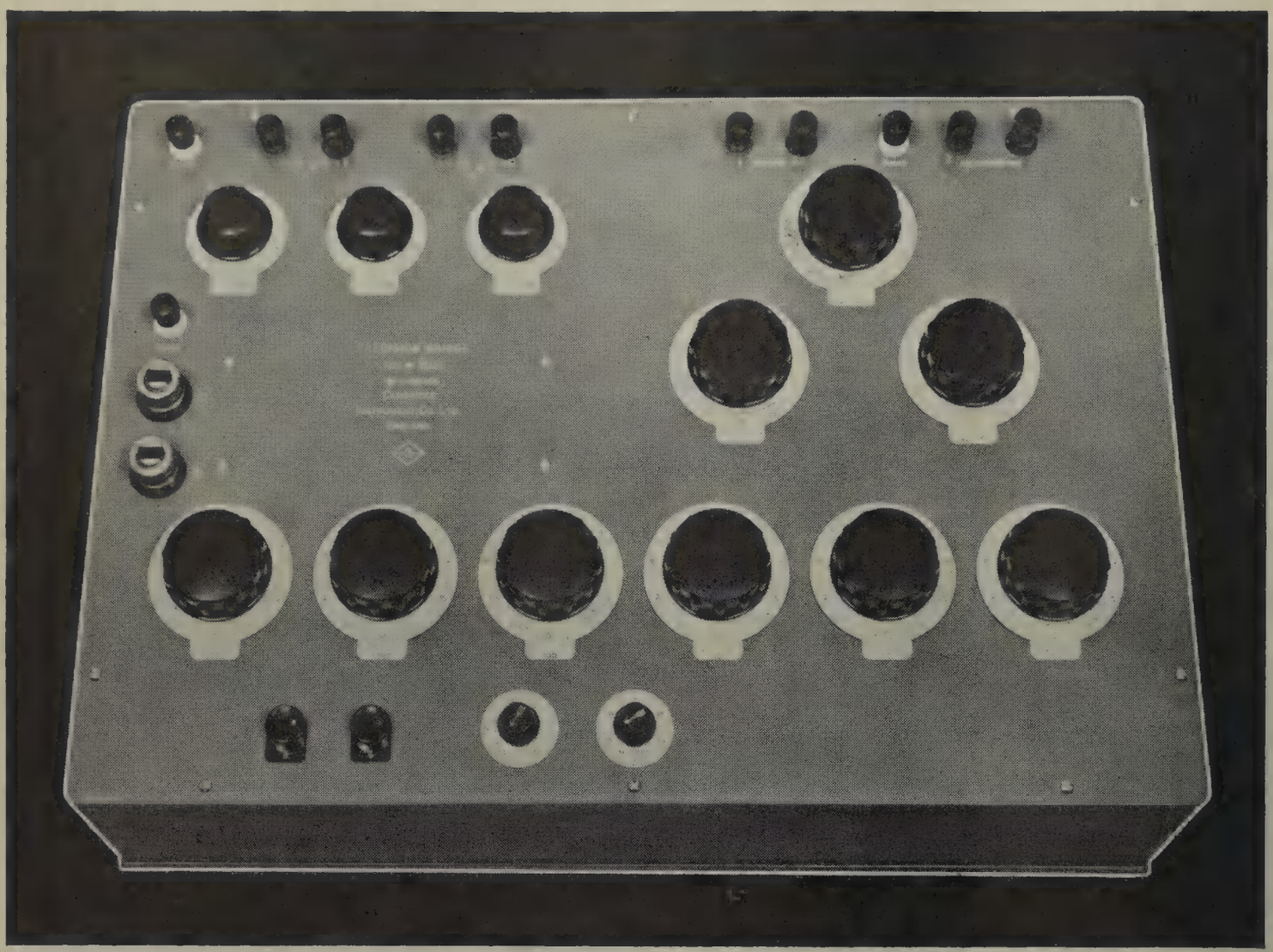

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as a shoulder on the monosome peak (see Fig. 3). 3 ml. of the 60-*S* shoulder was applied to a 53-ml. 20–33 per cent sucrose gradient and centrifuged for 10 h at 25,000 r.p.m. in the Spinco 'No. 25.2' rotor. The 60-*S* sub-units were resolved from contaminating monosomes and 40-*S* sub-units as a clear peak near the middle of this gradient (Fig. 4).

Poly-U was synthesized as previously described[17]. The preparation used in the experiments had $S_{20,w} = 9{\cdot}3$, and an average chain-length of about 300 residues, determined by measuring the inorganic phosphate released by bacterial alkaline phosphatase.

The very low polyribosome content of fraction *P*2–4 was reflected in a low level of endogenous protein synthesis in the cell-free system (Table 1) compared with fraction *P2H*. In contrast, poly-U-stimulated phenylalanine incorporation was rather higher in fraction *P*2–4, relative to *P2H*. Amino-acid incorporation by both fractions, whether endogenous or poly-U-stimulated, was completely dependent on the addition of ATP.

Polyphenylalanine synthesis by native and derived monosomes. Purified native monosomes were centrifuged through a 29-ml. sucrose gradient for 10 h (Fig. 1*a*). A single peak of optical density was found towards the bottom of the gradient. When the fractions were assayed for their capacity to synthesize polyphenylalanine in the cell-free system with added poly-U the amount of synthesis followed the optical density profile closely. In a parallel experiment derived monosomes were analysed in the same way with similar results (Fig. 1*b*). However, the specific activity of the derived monosomes was about 50 per cent greater than that of the native monosomes (Fig. 1 and Table 2).

In a previous report[17] it was suggested that polyphenylalanine synthesis by ribosome fractions containing a high proportion of polyribosomes was partly due to monosomes released from polyribosomes during the synthesis of haemoglobin in the cell-free system. Here the activity of derived monosomes is clearly demonstrated. The activity of both types of monosome in this experiment represents

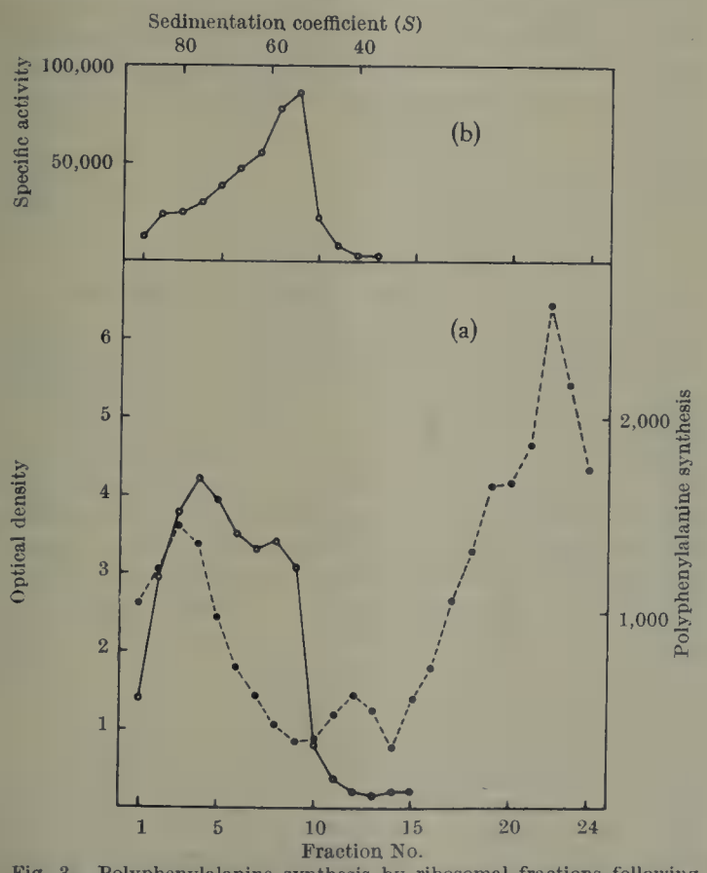

Fig. 3. Polyphenylalanine synthesis by ribosomal fractions following zone sedimentation. (*a*) Optical density at 260 mμ (broken line) and polyphenylalanine synthesis (solid line). (*b*) Specific activity. Direction of sedimentation from right to left. Zone centrifugation and assay procedure as for Fig. 2 except that sedimentation was for 10 h and 24 fractions of 1·25 ml. were collected

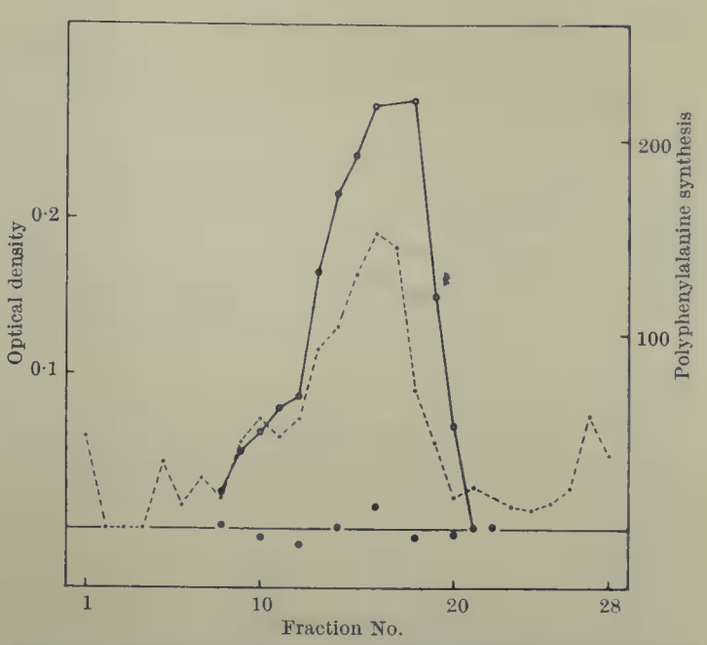

Fig. 4. Purification of native 60-*S* sub-units. Optical density (broken line) and polyphenylalanine synthesis as c.p.m. incorporated per 0·13 ml. in the absence (closed circles) and in the presence (open circles) of 40-*S* sub-units (5·6 μg). Direction of sedimentation from right to left. The preparation of 60-*S* sub-units is described in the text. Twenty-eight fractions of about 2 ml. were collected. Assay conditions were as for Fig. 1

the incorporation of less than 20 phenylalanine residues per monosome. If the average chain-length is about 200 (ref. 17), less than 10 per cent of these monosomes synthesize polyphenylalanine. The lesser activity of the native monosomes most probably indicates that a lower proportion is active; since the derivation of the two types was rather different, it is quite likely that one was more extensively inactivated than the other. However, the alternative interpretation, that the two types of monosome synthesize different lengths of polyphenylalanine, remains as a possibility.

Polyphenylalanine synthesis by native ribosomal sub-units. To determine which components of fraction *P*2–4 were active in polyphenylalanine synthesis, 1 ml. of the fraction was centrifuged for 5 h through a sucrose density gradient (Fig. 2), bringing the preponderant ribosome species, the monosomes, to the middle of the gradient. Setting the peak optical density at 80 *S*, the remainder of the gradient was calibrated by the approximation of Martin and Ames[20]. The 40-*S* ribosomal units were partially resolved, forming a shoulder at the trailing edge of the monosome peak. Endogenous protein synthesis was measured by incubating aliquots of the gradient fractions with ^{14}C-phenylalanine in the cell-free system in the absence of poly-U. No significant phenylalanine incorporation was observed. When poly-U was added to the cell-free system extensive phenylalanine incorporation occurred (Fig. 2*a*). A single peak of activity was found,

Table 2. Polyphenylalanine Synthesis by Sucrose Gradient Ribosome Fractions

Exp.	Fraction	Approximate sedimentation coefficient	Volume analysed (ml.)	Phenylalanine incorporation (c.p.m.)	Specific activity (phenylalanine residues per monomer equivalent)
Fig. 1*a*	4	80 *S*	0·2	673	9
	2–5 (av.)				10
Fig. 1*b*	5	80 *S*	0·2	818	13
	2–5 (av.)				14
Fig. 2	7	80 *S*	0·2	1,473	24
	9	50 *S*	0·2	1,499	62
Fig. 3	3	80 *S*	0·2	1,510	20
	8	60 *S*	0·2	1,364	62
	9	55 *S*	0·2	1,228	69
	7	60 *S*	0·1	658	42
	12	40 *S*	0·1	41	3
	7+12	60 *S*+40 *S*	0·1 each	2,358	80
Fig. 4	16	60 *S*	0·13	11	4
	40-*S* sub-units		0·07	20	3
		60 *S*+40 *S*	0·13+0·07	239	24

The fractions were assayed as described in the legend to Fig. 1. Specific activity is calculated from the optical density and phenylalanine incorporation of the fractions taking the optical density of a 1 mg per ml. solution of ribosomes to be 12 and the molecular weight of the monosome as 4×10^6 (ref. 21).

overlapping the optical density peak, but displaced towards the top of the gradient. The fraction with maximum activity lay between 60 and 70 *S*. A distinct peak of specific activity was also found (Fig. 2*b*), and the fraction with the highest specific activity lay still nearer to the top of the gradient, between 50 and 60 *S*. In the monosome region (80 *S*) the specific activity was three times lower. The peak fraction, fraction 9, incorporated approximately 60 phenylalanine residues per monomer equivalent (Table 2). Similar profiles, though showing lower levels of incorporation, were obtained when the fractions were incubated with smaller (8 μg) or greater (80 μg) amounts of poly-U.

In order to separate the various types of ribosome more completely, a similar sample of fraction *P*2–4 was centrifuged through the same gradient for 10 h (Fig. 3). Monosomes were found at the bottom of the gradient, clearly separated from the 40-*S* sub-units. The 60-*S* sub-units formed a shoulder on the trailing edge of the monosome peak (fractions 6–8, Fig. 3*a*). Haemoglobin was partly resolved from heavier contaminating proteins at the upper end of the gradient (fractions 15–24). Aliquots of the gradient fractions were incubated with poly-U and ^{14}C-phenylalanine in the cell-free system. Two peaks of activity were observed. The first tended to follow the monosome optical density profile, but merged into the second, which was maximum at about 60 *S*. The optical density and incorporation profiles resolved into a single peak of specific activity (Fig. 3*b*) with its maximum at about 55 *S*. Once again, the specific activity in the monosome region was about three times less than the maximum specific activity (Table 2).

The results show that the ribosomal sub-units are very active in polyphenylalanine synthesis. If both types of sub-unit were required, this would explain the fact that the highest specific activity is found between 40 and 60 *S*, where the two types of sub-unit probably overlap on the gradient. A direct demonstration of the requirement for both sub-units was obtained by mixing fractions from the 10-h gradient. When fractions 7 and 9 were incubated together, polyphenylalanine synthesis was 3·5 times greater than the sum of the two when incubated separately (Table 2). 40-*S* sub-units (fraction 9) showed little activity when incubated alone but the 60-*S* fraction, as isolated from the 10-h gradient, had considerable activity. Since the 60-*S* sub-units were poorly resolved, their activity might be due to contamination either by monosomes or by 40-*S* sub-units. To test this possibility the 60-*S* fraction was applied to a second gradient and centrifuged for a further 10 h. This separated the 60-*S* sub-units from contaminants which appeared as minor peaks on each side of the 60-*S* peak (Fig. 4). At this point the 60-*S* sub-units no longer showed any capacity for polyphenylalanine synthesis when incubated alone, but considerable activity was found when 40-*S* and 60-*S* sub-units were incubated together (Table 2). The profile of polyphenylalanine synthesis in the presence of 40-*S* sub-units followed the optical density profile of the 60-*S* sub-units quite closely (Fig. 4). The greatest specific activity observed in this experiment corresponded to the incorporation of 24 phenylalanine residues per monomer equivalent, which is much lower than the values found in the previous experiments (Table 2). This may be partly because in the assays of the peak tubes the ratio of 40-*S* to 60-*S* sub-units was about 5:1 on a molar basis. In fact, about 60 phenylalanine residues were incorporated per 60-*S* sub-unit present. A second factor may be the inactivation of purified sub-units. Variable inactivation (10–60 per cent) occurred when the sub-unit fractions were held at 0° C for the period of the second gradient run.

These experiments show that native ribosomal sub-units participate with poly-U in the synthesis of polyphenylalanine. Alone, each type of sub-unit appears to be inactive. The obvious inference is that the two types co-operate in the synthesis of each polyphenylalanine molecule. In these experiments sub-units incorporated up to 80 phenylalanine residues per monomer equivalent. Taking 200 as the chain-length of polyphenylalanine synthesized in the reticulocyte system[17], 40 per cent of the sub-units would be participating in polyphenylalanine synthesis, if each chain is synthesized by a single pair of sub-units. If more sub-units were required, the proportion of active sub-units would be higher, and the figure of 80 residues sets at 2·5 the upper limit to the number of pairs which might be involved in the synthesis of a single polyphenylalanine molecule. These arguments are based on the assumption that a given sub-unit can participate in the synthesis of only one polyphenylalanine molecule.

Discussion. The native ribosomal sub-units described here have sedimentation coefficients of about 40 and 60 *S* (ref. 7), as have the derived sub-units prepared by the disaggregation of monosomes[5]. Under conditions which have been reported to effect the reaggregation of derived sub-units[5], native sub-units are completely stable[7]. Furthermore, native sub-units are isolated from the cell lysate under conditions in which monosomes are stable. These facts suggest that there is an inherent structural difference between native and derived sub-units, although it is clear that before a definitive statement can be made the differently derived sub-units must be compared side by side.

It has not yet been determined whether the native sub-units are in sub-unit form during polyphenylalanine synthesis, or whether they are aggregated together to form monosomes or higher order structures.

The specific incorporation figures for native monosomes and sub-units (Table 2) allow an estimate to be made of the relative contributions these fractions would make to total polyphenylalanine synthesis in cell-free systems. Native sub-units make up about 15 and monosomes about 60 per cent of the total ribosomal material in the clarified lysate (unpublished experiments). In the lysate, about 60 per cent of polyphenylalanine synthesis would be due to both native and derived monosomes, and about 40 per cent to sub-units. The same would be true of purified systems using ribosome preparations which had been sedimented long enough to precipitate all sub-units, that is for 3–4 h at 78,000*g*. Where shorter sedimentation times are used the proportional contribution of the monosomes will be greater. The same will be true of pre-incubated systems, regardless of the method of preparation, since pre-incubation under conditions favouring protein synthesis leads to the disappearance of sub-units[7].

E. coli ribosomes have rather different properties than reticulocyte ribosomes, and it is not at all clear what homologies, if any, exist between ribosomes and sub-units from the two organisms. Green and Hall[22] isolated sub-units from an *E. coli* lysate which failed to aggregate under conditions which led to the aggregation of derived *E. coli* sub-units. It is possible that these correspond in some way to the native reticulocyte sub-units, but it is not known whether they are active in polyphenylalanine synthesis. Gilbert[13], and Okamoto and Takanami[23], prepared two types of ribosomal sub-unit from *E. coli* and showed that when incubated together, but not when incubated alone, they would synthesize polyphenylalanine. In each case the sub-units were prepared after the complete dissociation of all monosomes. It is therefore not clear whether the activity observed was due to the derived or the native sub-units.

The question arises of whether native sub-units or monosomes or both are responsible for the initiation of endogenous polypeptide chains, the chains of the globin molecule in the present case. Recent evidence[7], much of it indirect, indicates that sub-units are required. In certain circumstances a simple one-to-one relationship was demonstrated between the number of sub-unit pairs available and the number of chains initiated. Strong indications were found that monosomes do not initiate endogenous polypeptide chains.

On the other hand, Hardesty *et al.*[10] and Lamfrom and Knopf[24] have suggested that monosomes do initiate globin chains. Under the conditions used by these authors for the preparation of monosomes, sub-units would have been disaggregated. It is likely, however, that the monosomes were not resolved from the disaggregated sub-units. The monosomes used by Goodman and Rich[25] in reaching the same conclusion from experiments with an ascites cell system were more thoroughly purified. However, it is not known whether or not ascites cell native sub-units would be aggregated under the conditions of isolation.

The results presented here support the idea that sub-units are active in polypeptide initiation; but at first sight they tend to contradict the conclusion that monosomes are inactive. Monosomes are clearly shown to be active in polyphenylalanine synthesis, although less so than sub-units. However, it is by no means certain that the properties of poly-U and the endogenous informational RNA are the same. Secondly, in the experiments reported here the ratio of poly-U molecules to ribosomes was of the order of 100 : 1. In the endogenous situation it is likely to be less than 1 : 1. The reactivity of the ribosome fractions with poly-U may not be a certain index of their behaviour either within the cell or in the cell-free system without added polynucleotide.

I thank Mrs. Norma Robertson for her assistance, and Mr. B. B. Cohen for his advice. This work was supported by grants from the Medical Research Council.

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INTERMEDIATE STATES IN THE PRECIPITATION OF HYDROXYAPATITE

By Prof. E. D. EANES, Dr. I. H. GILLESSEN and Prof. A. S. POSNER
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TO distinguish between the physico-chemical and physiological factors underlying the formation of biological hydroxyapatites, investigations have been undertaken in this laboratory on the ageing of aqueous preparation of synthetic calcium phosphates. In these studies, chemical and X-ray diffraction analyses are being employed to elucidate certain aspects of the nucleation and growth dynamics of the emerging solid phase under a wide variety of experimental conditions. The present article describes the precipitation behaviour of these calcium phosphates under conditions of high *p*H and high initial concentrations of reactants, and gives a description of some of the alterations which occur in the physical and chemical properties of the solid phase while in contact with the preparative solution.

To minimize the formation of more soluble acid phosphates, the following hydroxyapatite preparation, a modification of one proposed by Hayek and Stadlmann[1], was employed: 0·250 molar solutions of $(NH_4)_2HPO_4$ were rapidly added while stirring to 0·750 molar solutions of either $Ca(NO_3)_2.4H_2O$ or $CaCl_2.6H_2O$ to achieve a final phosphate concentration of 0·15 molar in the Ca/PO_4 molar ratio 1·71. All solutions were brought to a *p*H value of 10·5 with concentrated NH_4OH before mixing. The reactions were carried out at 25° C in closed systems to minimize CO_2 absorption. Constant stirring kept the slurry in intimate contact with the reaction solution for periods up to 2 weeks. Samples of the reaction slurry were withdrawn at fixed intervals after initial mixing for chemical, *p*H and X-ray diffraction analyses. To avoid changes in the solid during the drying of the slurry, all samples were lyophilized. The samples chosen for chemical studies were washed four times with ammoniated wash water (*p*H 10·5) before freeze-drying and then analysed for Ca by standard EDTA titration techniques and for PO_4 by the differential spectrophotometric technique of Gee and Deitz[2]. The supernatant and washings were also analysed for Ca and PO_4.

X-ray diffraction patterns of the samples were recorded on an electronic diffractometer equipped with a scintillation counter and a pulse-height analyser. Nickel-filtered copper $K\alpha$ radiation was employed. The scattering region 24–36°2θ was principally surveyed; this portion of the diffraction pattern includes the main hydroxyapatite diffraction profile. Because of extensive overlaps, only the 002 peak at 25·8°2θ was sufficiently resolved for line-breadth measurements. The width at one-half the maximum height of this peak, $\beta_{1/2}$, was measured in degrees 2θ and corrected for instrumental aberrations according to the method of Warren[3]. The quantity, $1/\beta_{1/2}$, which is proportional to the mean crystallite size and/or degree of perfection in the direction along the *c*-axis of the crystals was taken as the measure of the crystallinity index. Since it was observed qualitatively that variations in the degree of resolution of the remainder of the diffraction pattern parallel the changes in the 002 reflexion, this crystallinity index can be taken as representative of the crystal as a whole.

The initial solid phase, which separated immediately on mixing of the reactants, was 'non-crystalline' inasmuch as the diffraction patterns showed no discrete diffraction peaks (see Fig. 1*A*). The molar Ca/PO_4 ratio of this amorphous phase after washing was well below the 1·71 ratio of the overall reaction system and was found to be dependent on the Ca salt used in the preparation. The molar Ca/PO_4 ratio was remarkably consistent for any given salt; a ratio of 1·52 was found when the nitrate solution was used, a value of 1·46 was observed when the chloride solution was used. At most only 90 per cent of the total Ca available in solution was incorporated into the amorphous phase while the unincorporated Ca either remained in solution or was washed out of the precipitate. No detectable amount of PO_4 was found in either the reaction solution or the wash waters after solid formation.

X-ray diffraction patterns taken on the unwashed, freeze-dried product, as well as patterns taken directly of

the slurry, centrifuged to remove excess water, established that the 'non-crystalline' character of the precipitate persisted in contact with the precipitating solution for several hours before completely transforming into a poorly crystalline hydroxyapatite (Fig. 1*B*). The observable conversion of the pre-apatitic phase into hydroxyapatite did not occur abruptly, but extended over a period of about 2 h. Measurements made on the 002 diffraction peak of samples collected during this period revealed that even though there was a continuous five-fold increase in relative peak area, indicative of a five-fold increase in converted apatite, the diffraction breadth remained unchanged at $1/\beta_{1/2} = 1{\cdot}44/^{\circ}2\theta$ ($\sigma = \pm 0{\cdot}04$), a value smaller than normally found in poorly crystallized bone apatite[4]. When the pre-apatitic phase has fully converted, as evidenced by the constancy of the 002 diffraction peak area, a gradual sharpening of the 002 reflexion was observed ($1/\beta_{1/2} = 1{\cdot}75/^{\circ}2\theta$ at 25 h, $1/\beta_{1/2} = 2{\cdot}39/^{\circ}2\theta$ at two weeks).

Increase in the Ca/PO_4 molar ratio of the solid and corresponding decreases in the concentration of solution Ca occurred concomitantly with the crystallographic conversion. By the end of two weeks the Ca/PO_4 molar ratio of the solid had increased to $1{\cdot}67 \pm 0{\cdot}01$, equalling that for pure hydroxyapatite and the Ca in solution had been considerably reduced from 10 per cent to about 2 per cent of the total Ca available in the solution at the start of the reaction. As before, no phosphate was detected in the reaction solution. An overall Ca/PO_4 molar ratio in slight excess of 1·67 was employed in these experiments to ensure that the final ratio of the solid was independent of the availability of solution Ca. The *p*H of the reaction solution, on the other hand, dropped from 10·5 to a value of 9·8 immediately on mixing and remained at this value during the entire period of crystallization. The crystal-chemical events described here were greatly accelerated when the reaction was conducted at 30·5° C instead of 25° C.

The ageing of freshly precipitated calcium phosphate as prepared under conditions described in this article can be divided into three stages (Fig. 2). The first stage, which commences immediately on mixing and continues for about 5 h, is characterized by an amorphous-like diffraction pattern which does not undergo any overt changes. Stage two is marked by the progression from a barely discernible apatitic profile superimposed on the amorphous pattern to a fully developed apatite pattern with the complete disappearance of any underlying amorphous features. The third stage covers the period following total conversion of the amorphous phase to a crystalline apatite and is characterized by the constancy of the 002 peak area. This final stage commences about 7 h after the reaction is initiated, and continues indefinitely.

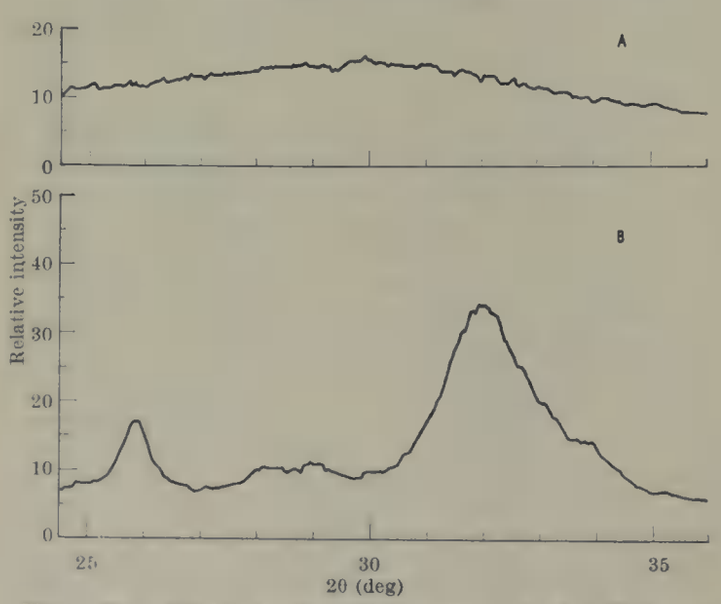

Fig. 1. X-ray diffraction patterns of (*A*) non-crystalline and (*B*) crystalline (apatitic) calcium phosphates (copper $K\alpha$ radiation)

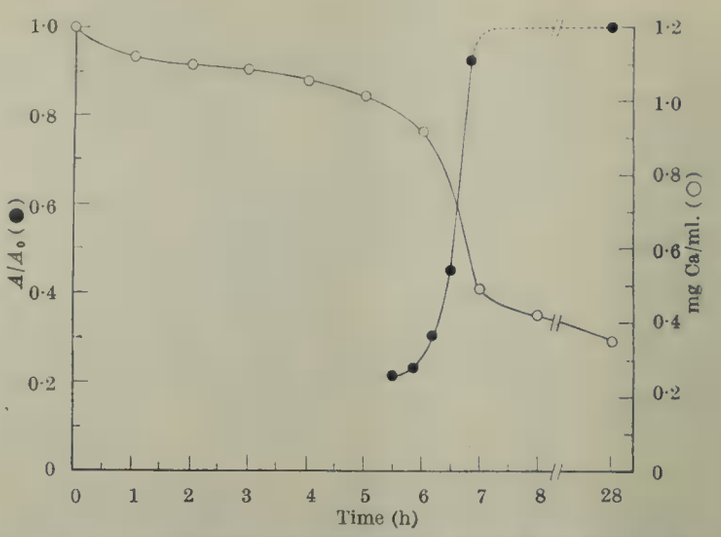

Fig. 2. The curve connecting the closed circles shows the per cent conversion of non-crystalline to crystalline calcium phosphate (A/A_0) as a function of time. The plot connecting the open circles indicates the drop in solution Ca concentration (mg Ca/ml.) with time

Despite the apparent constancy in the diffraction pattern, the first stage is not chemically static. Slight but significant decreases in solution Ca occur during this period (from 1·20 mg/ml. to 1·02 mg/ml.; Fig. 2). Moreover, the demarcation between stages one and two is arbitrarily based on the initial appearance of a discrete apatite diffraction pattern. Since the 002 peak area at the beginning of the second stage is already about 1/5 the value achieved at maximum conversion, it is clear that about 20 per cent of the amorphous material has been converted to the crystalline phase by the time stage two can be detected. Thus, crystallographic as well as chemical changes are taking place in this initial period. Nearly coincident with the emergence of a discernible diffraction profile, however, is a more rapid decrease in solution Ca-levels (from 1·02 mg/ml. at the beginning of stage two to 0·42 mg/ml. at the end; Fig. 2). In addition, the 002 diffraction peak, once discernible, proceeds to develop at a rate commensurate with the accelerated rate of fall in solution Ca-levels, so that the remaining 80 per cent of pre-apatite converts over a much shorter interval than the initial 20 per cent. One can conclude, then, that while no fundamental distinction may exist between stages one and two, a distinction between rates of conversion can be made with validity, with the crystal phase formation taking place at a much accelerated pace in the second stage than in the first. The constancy of the 002 crystallinity values up to the end of the second stage suggests that the rate of conversion of the amorphous phase in stage two depends on the nucleation of new crystals rather than the growth or perfection of pre-existing crystals.

The most characteristic feature of the third stage is the gradual increase in crystallinity. The improvement in hydroxyapatite crystallinity during this period is typical of the gradual enlargement of mean particle size of many low-solubility precipitates as a result of re-precipitation in solution. This growth of the precipitate, known as Ostwald ripening, is generally attributed to the dissolving of the smaller, more soluble crystals and re-deposition of the dissolved material on the growing faces of the larger, less-soluble crystals[5]. No doubt this re-crystallization is in progress from the earliest stages of the conversion, but the time interval in stage two is too short for this gradual increase in crystal dimensions to have a measurable effect on the crystallinity values measured during this period. The gradual decrease in solution Ca from 0·42 to 0·20 mg/ml. during this final stage indicates that a perfection of the crystalline phase parallels the crystal growth.

To describe the initial phase as 'non-crystalline' is to imply only that there has been insufficient development of

a regular, repetitive, spatial array of ions to define a crystalline structure. This does not preclude the possibility of localized order and structure. Indeed, the fact that the molar Ca/PO_4 ratio does not vary under a given set of initial conditions suggests a chemically well-defined local structural unit.

The formation of 'non-crystalline' calcium phosphate is a kinetic phenomenon. The rapid mixing of the reaction solutions creates strong interactions between Ca^{++} and $HPO_4^{=}$ ions leading to irregular co-ordination complexes large enough in size to separate from solution. The subsequent temperature-dependent transformation into apatite indicates that this initial phase is isothermally metastable with respect to the more ordered apatite configuration. In addition, the sigmoid-shaped relationship of the transformation with time strongly suggests that the conversion mechanism is autocatalytic. It is possible that the interfacial surfaces of the emerging crystalline phase act as sites for heterogeneous nucleation, thereby accelerating the rate of conversion. In this regard, the 'non-crystalline' calcium phosphate remains stable indefinitely if kept dry, pointing out the necessity for water for the process of nucleation to take place in this system.

This work was supported in part by U.S. Public Health Service grant *DE*-01945 from the National Institute of Dental Research.

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EFFECTS OF SEEDING ON THE CONDENSATION OF ATMOSPHERIC MOISTURE IN NOZZLES

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A PRELIMINARY investigation has been made of the effects of inorganic smokes on the condensation of atmospheric moisture in the wind tunnel. The operation of the tunnel, and earlier results obtained with unadulterated air, have been described elsewhere[1,2]. Theoretical calculations had indicated[3,4] that chance impurities such as dust particles could not interfere detectably with homogeneous nucleation of the moisture unless present in concentrations of 10^6 particles per c.c. or greater. It was thought desirable to test this experimentally.

In the experiments, smokes of AgCl, AgI, CdI_2, CuBr, KI, and PbI_2 were generated in the atmospheric inlet of the wind tunnel by evaporation of the compounds on 30-ohm heater coils of nichrome wire. Evaporation rates at various supply voltages were measured by the periodical weighing of test sections of the coils loaded with salt. From 90 to 150 V, the useful range for smoke generation, evaporation was steady and reproducible with a rate of the order of 5 μg/sec/cm of coil.

Smoke particles were collected for examination by supporting microscope slides and electron microscope grids in the air flow about 10 cm downstream of the smoke generator. The deposit which built up on the support of the pressure probe in the test section was also sampled. Methods of examination included optical and electron microscopy and X-ray diffractometry. Microscopy disclosed a variety of particle sizes. At high heater voltages particles of up to 1μ diam. were present, but at low voltages only sub-micron particles occurred. A useful idea of the relative abundance of small and large particles could be gained with the optical microscope, but the electron microscope was more informative about the structure. In AgI smoke generated at 140 volts there were considerable numbers of 1μ coagula containing up to 20 particles of 0·01μ diam. or less. At 120 V there were fewer large aggregates, sizes below 0·1μ being more frequent. Coagula were rare in smoke produced at 100 V and most of them consisted of only two particles. Nearly all the remaining particles were less than 0·01μ in diam., and even the aggregates were smaller than 0·1μ. It is evident that the total concentration of particles in the smokes increased rapidly as the voltage was lowered because the mass evaporation rate was much the same at all voltage settings. In cloud chamber experiments it has also been found that AgI aerosols tend to contain coagula when the generating temperature is high[5].

The course of condensation was followed in two nozzles (Nos. 4 and 6) by measuring the static pressure with an axial probe[1]. The air temperature at the inlet was 24° ± 2° C. Mass flow rates of dry air in the nozzles were 972 and 1,110 g per sec respectively. The maximum speed of dry air in the fast nozzle (No. 4) occurred at a Mach number of 1·8. The temperature gradient in this nozzle was approximately 14° C per cm. In the slow nozzle (No. 6) maximum speed was attained at a Mach number of 1·6, the temperature gradient being about 2° C per cm. Other experimental details are given in Table 1.

Typical results are shown in Figs. 1 and 2, where the ratio of the local static pressure, p, to the supply total pressure, p_{01}, is plotted against the position of the probe along the nozzle axis. Positive deviations from the isentropic curves, that is, 'humps', are the result of heat evolved during the condensation of moisture. A level of heat addition exists above which there occurs a normal shock, and this gives rise to a marked peaking in the pressure curves. This critical heat addition depends on the Mach number of the flow in the vicinity of the condensation zone[1,2]. The positions of shocks in supercritical runs are indicated in the figures.

Seed addition caused the hump to be flattened and displaced downstream. The effect was produced by all compounds and was most drastic in the slow nozzle (curves 644, 645 in Fig. 1 and curves 592, 594 in Fig. 2). In both nozzles the extent of the flattening and displacement decreased as the humidity was lowered. Increase in the voltage of smoke generation also caused a decline in the effect (curves 593, 594 in Fig. 2), presumably through the reduction in the total concentration of seed particles.

Table 1. Experimental Details

Exp.	Nozzle	Relative humidity* (%)	Seed	Heater voltage
601	4	27·0	—	—
602	4	27·0	AgI	100
644	4	48·0	—	—
645	4	48·0	AgI	100
663	4	74·0	—	—
664	4	74·0	AgI	110
674	4	80·5	KI	110
582	6	19·0	—	—
581	6	19·0	AgI	120
578	6	34·0	—	—
698	6	34·0	CuBr	110
592	6	45·5	—	—
593	6	45·0	AgI	140
594	6	45·5	AgI	90

* At atmospheric inlet.

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Conditions for the onset of the seeding effect could not be determined exactly because of the lack of precise control over humidity and smoke concentration. It began at an inlet moisture-level between 27 and 48 per cent relative humidity in nozzle 4 and between 19 and 45 per cent relative humidity in nozzle 6 (Figs. 1 and 2). The exact level was probably highest in the fast nozzle (No. 4). Irregular pressure readings obtained at 34 per cent relative humidity in nozzle 6 (curve 698) were possibly connected with the threshold of the seeding effect, but this needs clarification. From the mass flow rate of air and the evaporation rate of the smoke compound it is calculated that about $10^3/D^3$ smoke particles per c.c. of air were present in the intake, where D is the particle diam. in μ. For the given sizes this means that the seeding effect was observable at concentrations of 10^8 particles per c.c. and above.

The shape of the residual condensation hump in a seeded run, and the location and visual structure (schlieren pattern) of the shock wave (if any) in the hump, were closely similar to those in an un-seeded run at a lower humidity (curves 663, 674 in Fig. 1 and curves 578, 594 in Fig. 2). This leads to the following conclusions. In a seeded run moisture condenses on the smoke particles upstream of the hump, the quantity so condensed depending on the smoke concentration and the flow conditions imposed by the nozzle. The hump is caused by spontaneous condensation, and this process is substantially independent of the preceding heterogeneous process.

It is possible to compare in a rough way the amounts of catalytically and spontaneously condensed moisture by matching humps for seeded and un-seeded runs. Two suitable examples occurred fortuitously in the present experiments. Curves 663 and 674 (Table 1) show that about one-fifth of the water condensed out on the KI nuclei in nozzle 4 at an inlet humidity of 80·5 per cent relative humidity. The moist air took 120 μsec to travel down the nozzle from the point at which saturation was reached to the point where the hump began, cooling through 57° C on the way. Curves 578 and 594 show that in nozzle 6 at an initial humidity of 45·5 per cent relative humidity some two-fifths of the moisture condensed out on the AgI. In this case the air cooled through 28° C in 400 μsec while flowing between the saturation point and the hump. Bearing in mind the fall in pressure, p/p_{01} decreasing from 0·9 to 0·5 in the first example and from 0·5 to 0·4 in the second, it is seen that termination of the heterogeneous process by the homogeneous occurs sooner the more rapid the expansion.

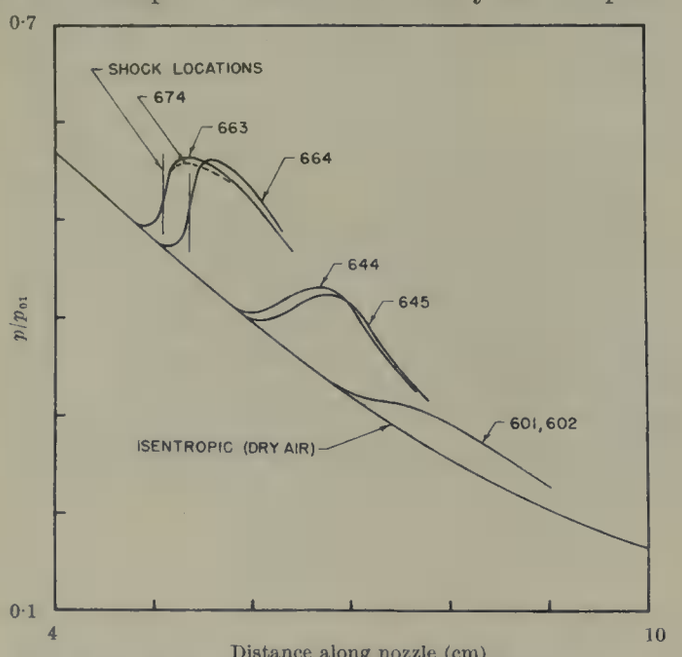

Fig. 1. Static pressure ratios for fast nozzle (No. 4: sonic point at 4·5 cm). Curve numbers refer to experiments in Table 1

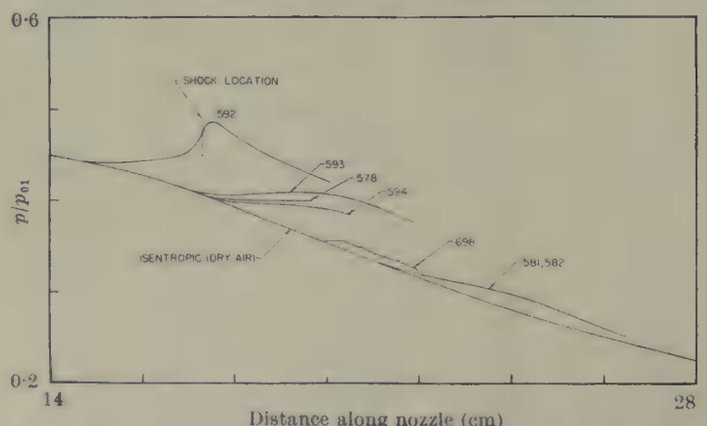

Fig. 2. Static pressure ratios for slow nozzle (No. 6: sonic point at 8·0 cm). Curve numbers refer to experiments in Table 1

The fact that the isentropic curve is indistinguishable from the moist air curve in the region where condensation occurs only on the seed shows that this condensation is a far more gradual process than the homogeneous one. In the first process, a fixed number only of artificially established nuclei are available, while in the second much larger numbers of natural centres are possible. If apart from the lowering of available moisture the presence of 10^8 seed particles per c.c. has little or no effect on spontaneous condensation, as appears likely from the present results, numbers of self-nuclei exceeding 10^8 per c.c. must build up very rapidly in the beginning of the hump. In fact, since growth of seeded condensate particles is supplanted in this region, multiplication of spontaneous centres rather than their growth is indicated. While nucleation continues in the hump the homogeneous condensate probably consists of particles smaller than the seed (that is, less than 0·01μ diam.).

The discrepancy between the maxima in curves 663 and 674 suggests that at high-supply humidities the presence of seeded condensate may interfere with homogeneous nucleation. It should be borne in mind, however, that the possibility of disturbance in the flow by the condensation shocks present in these runs throws some doubt on the accuracy of the pressure readings downstream of the shocks. The evaluation of any interference between the condensation processes from pressure data alone would therefore be difficult for super-critical runs.

A reversal of the trend towards sharper humps with higher humidities was observed in un-seeded nozzle expansions of very humid air. This was gas-dynamical in origin[2], but it is still possible that the inversion shown by curves 664 and 674 was contributed to by differences in AgI and KI as seeding materials. Apart from this, there were no obvious differences in any of the compounds studied.

The high activity of AgI as a catalyst for ice nucleation in water and water vapour has been attributed[6] to the close matching of the crystal structures. This proposal was recently questioned[7] on the basis of the limited adsorptive power of AgI for water vapour and the role of surface topography[8] in heterogeneous ice nucleation. Experiments on the crystallization of ice from water under pressure have since confirmed that for a given substrate material the polymorphic form most closely resembled in structure by the substrate is preferentially nucleated, even when it is metastable[9].

The chemical influences on the nucleating properties of different compounds are still not clear, and it may be asked whether the results recorded here lead to any new conclusions. The central problem would seem to be whether an ice or a water nucleus first forms on the seed. The X-ray evidence showed that AgI smokes containing large particles were crystalline with the hexagonal structure. Distinction between the hexagonal and cubic forms was obscured for small-particle smokes by the poor resolution of peaks in the diffractometer recordings. Manson[10], whose mean particle size corresponded to the largest single particles in the present smokes (0·1μ),

observed that the hexagonal AgI structure gradually replaces the cubic as the volatilization temperature is increased. Morphological changes accompanied by an increase in particle size have since been observed in a variety of condensation aerosols on raising the temperature of generation[5,11]. It may be concluded that the hexagonal form of AgI was more abundant in the high-temperature smokes. If the nozzle condensate begins as two-dimensional ice nuclei, the greater seeding power of AgI smoke generated at low temperatures would imply that the effect of a decrease in the proportion of hexagonal material was outweighed by the increase in the particle concentration. Alternatively, the topographical requirements for adsorption are likely to be a good deal less stringent generally than those for ice nucleation. The apparent similarity in heterogeneous activity of all the compounds, with the implied unimportance of equilibrium crystal structure, might be more easily explained if, under the conditions of a nozzle expansion, the initial step was the formation of a water layer on the seed.

To recall our main conclusions, it is confirmed that insufficient heterogeneous nuclei are present in normal air to interfere with the homogeneous condensation of water vapour in the wind tunnel. Seeding with foreign particles in concentrations of 10^8 per c.c. or above has an effect when the humidity in the intake is raised above a certain level dependent on the nozzle flow. Under moist climatic conditions, reduction of humidity by seeding makes it possible to study homogeneous condensation in shock-free flow. The experiments provide some idea of the conditions under which spontaneous and catalysed condensation of water vapour occurs competitively. To put this on a quantitative basis to test, among other things, theories of nucleation and growth in aerosols requires further work to establish exact relations between seed concentration, inlet conditions, and flow variables.

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METAL CONCENTRATION PROFILES IN OXIDE SCALES ON NICKEL–10·9 PER CENT COBALT ALLOY

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FOR many years it has been widely accepted that when nickel is oxidized in oxygen at high temperature a simple, single-phase, single-layer scale of NiO grows on its surface according to a parabolic relationship. The NiO is supposed to be a metal-deficit, *p*-type semiconductor growing by the outward movement of electrons and Ni^{2+} ions to form fresh oxide at the scale/oxygen interface[1]. Cobalt behaves similarly above 900° C, but the CoO scale grows much more rapidly[2], an observation in agreement with measured self-diffusion coefficients of the metal ions in their respective oxides[3–5]. Recently, however, the situation has been shown to be more complex because double-layer scales, each consisting of the single oxide, have been found on both metals[6–13,15], with the consequence that ideas of oxygen ion or gas movement through parts of the scale have been invoked. The present state of knowledge has recently been reviewed elsewhere[14].

Now, nickel–cobalt alloys, which are homogeneous and single-phase, also produce either single or double layers of a single oxide solid solution (Ni,Co)O when oxidized above 900° C—cobalt being only slightly preferentially oxidized[14,15]. This almost unique alloy oxidation situation provides an excellent opportunity for following the concentration profiles in alloy and scale directly by electron-probe microanalysis at various stages of growth. The method has problems of its own, but it has some advantages over marker experiments and certain tracer techniques. Such an investigation is reported here because it provides new precise evidence for oxidation of the alloy, with possible interpolation of the results to the case of the pure metals.

Annealed specimens of Ni–10·9 per cent Co, of purity in excess of 99·95 per cent, $2{\cdot}5 \times 0{\cdot}6 \times 0{\cdot}066$ cm, were electropolished at 0·7 amp/cm² (2·5 V) in 60 per cent sulphuric acid (s.g. 1·6) at 30° C for 1 min. After drying, they were immediately oxidized in pure oxygen in a silica-spring thermobalance for 15, 30 min and 1, 2, 3, 6, 10, 24 and 48 h respectively at 1,000° C, followed by cooling over a 15-min period to minimize scale cracking. Other short-term specimens were simply exposed in flowing oxygen in the hot zone of a furnace for 10 and 15 min respectively without slow cooling. The latter specimens were examined directly in plan in the microanalyser whereas the former were prepared metallographically in cross-section before analysis.

All the specimens gave reasonably reproducible growth kinetics with a close, if not quite exact, agreement with the parabolic relationship (Fig. 1). The growth rate was the same on specimens given a 5-sec etch in 50/50 (v.) glacial acetic acid/nitric acid after electropolishing and was only slightly less on specimens mechanically polished on 600 g silicon carbide paper. The mean parabolic rate constant for the electropolished specimens was $4{\cdot}9 \times 10^{-10}$ $g^2cm^{-4}sec^{-1}$.

Each specimen displayed in section a uniform, single-layer single-phase oxide, generally adherent to the alloy

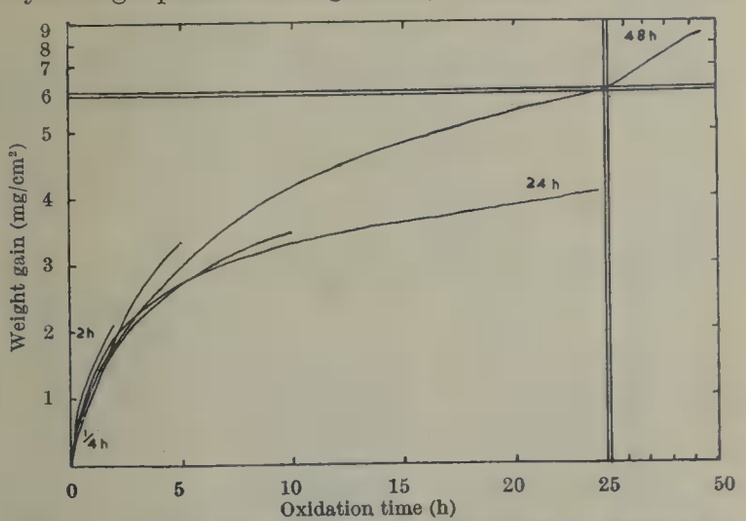

Fig. 1. Weight gain/time curves for Ni–10·9 per cent Co oxidizing in oxygen at 1,000° C

and with no observable colour change through its depth (Fig. 2). Internal oxide particles were present, but were few in number. The outer surfaces of all specimens examined in plan were blue–grey with characteristic topographical features which will be discussed elsewhere. It is likely that oxide colour in this system is characterized more by grain size than by chemical composition.

Typical microanalyses for the thin scales examined in plan are given in Fig. 3. Since for both films the total wt. per cent metal curves would lie within a few per cent of 78 per cent, the X-ray source was clearly entirely enclosed within the scale over the kilovoltage range used, namely 15–35 kV. As the kilovoltage was reduced, the source became located to a greater extent in the outer region of the scale (the source sizes giving 95 per cent of the radiation were about 0·8, 1·1, 1·5, 1·8 and 2·2μ at 15, 20, 25, 30 and 35 kV respectively). The results prove that when the surface scale was formed on this alloy it was immediately enriched in cobalt. They also appear to show that this enrichment at the outer surface was greater after 15 min than after 10 min. This could be genuine, but an alternative explanation is that at all

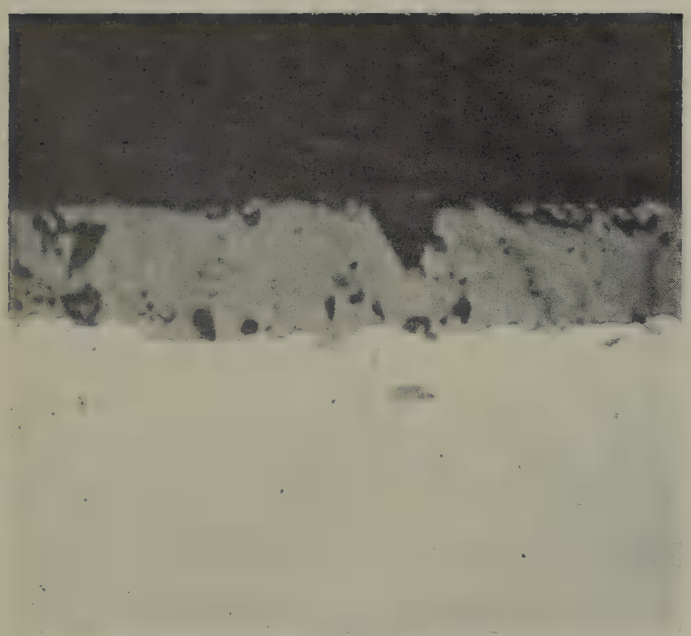

Fig. 2. Typical cross-section through scale on Ni–10·9 per cent Co oxidized in oxygen for 3 h at 1,000° C (×1,000)

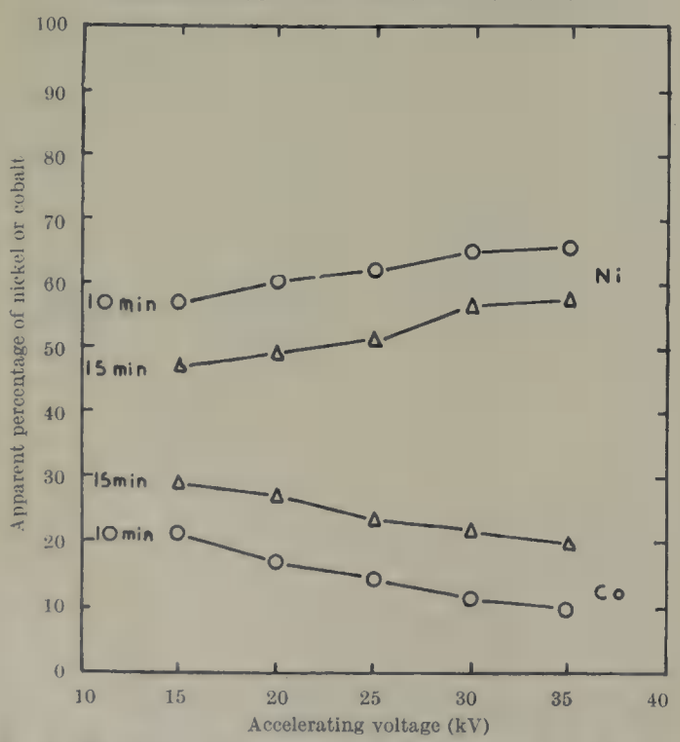

Fig. 3. Examination of thin scales on Ni–10·9 per cent Co in plan by electron-probe microanalysis

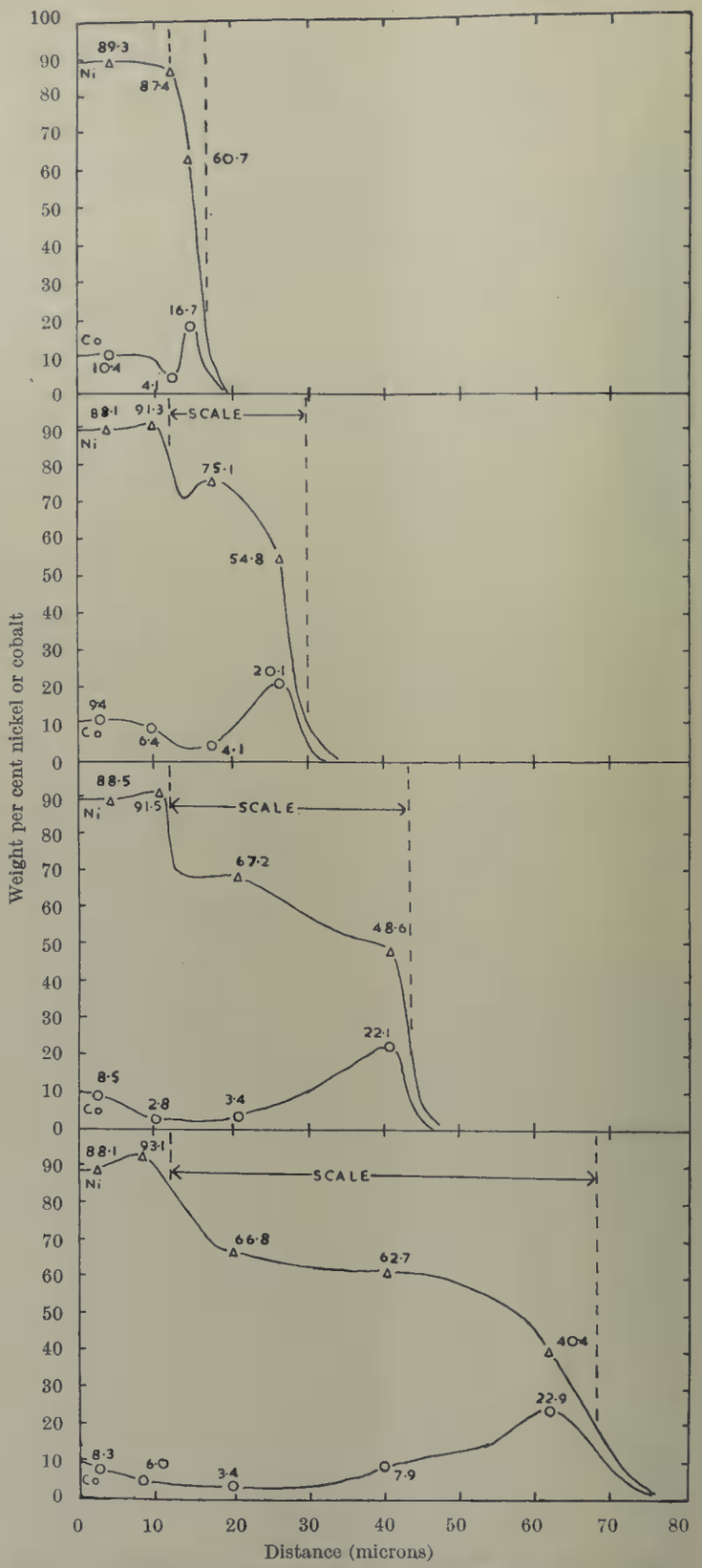

Fig. 4. Distribution of nickel and cobalt through alloy and scale for Ni–10·9 per cent Co oxidized in oxygen at 1,000° C. Top to bottom: 15 min, 2 h, 24 h, 48 h

kilovoltages more of the nickel-rich inner region was incorporated in the source for the 10-min specimen because the outer region was quite thin. Plan measurements of this kind permit a more accurate analysis of the outer micron or so of scale than do measurements in section because of their greater resolution.

This general situation is confirmed in Fig. 4, where concentration profiles in cross-section at various stages of oxidation are presented. The profiles are smooth with a pronounced cobalt peak, apparently containing from 16·7 to 22·9 per cent cobalt, near the outer surface. This value is probably rather low, due to relief polishing problems near the outer interface and to the lower resolution of the microanalyser under these conditions

(measurements were made at 25 kV). An obvious observation is the similarity in shape of the cobalt and nickel profiles in the films of different thickness, almost as though the cobalt peak were merely growing a longer tail extending to the alloy/oxide interface. Closer scrutiny, however, shows that the outer cobalt-rich peak was in fact growing in thickness and possibly in cobalt content, although the latter effect was small and obscured for the reasons given above. Measurement of the areas under the curves shows that the percentage cobalt in the scales lay between 20·4 and 11·1 per cent, but this varied in a slightly irregular manner with time, probably due to minor measuring errors, and is being studied further. The alloy was depleted in cobalt and enriched in nickel over a small distance.

It is possible to draw several other conclusions from this investigation, some definite but others more tentative and requiring further thought and experiments which are in progress:

(1) The reasonable agreement with a parabolic growth relationship may not be particularly meaningful because of the large compositional variations through the scales. Presumably the cobalt-rich regions offer less resistance to the movement of the diffusing species than the nickel-rich regions because they probably contain a larger percentage of cation vacancies. This is further proof of the need to be careful when carrying out kinetic studies of alloy oxidation. On the other hand, the present situation is really an extreme case of one previously reported for the oxidation of pure cobalt[5], where it was inferred from marker experiments that the parabolic relationship held even for a non-linear vacancy distribution through CoO. Since the compositions of scale in contact with the alloy and atmosphere respectively did not change much with time in the present case, at least after the first hour and probably from a much earlier stage, it is perhaps reasonable to conclude that the cation vacancy concentration difference across the scale remained constant, which was the only requirement of this type specified in Wagner's original derivation of the parabolic relationship.

(2) As mentioned earlier, at first sight it appears that after the initial cobalt-rich peak was rapidly established, it was pushed further and further from the alloy, fresh oxide being formed near the alloy/oxide interface. This conclusion would support early ideas[16,17] and more recent revolutionary ones[18] for oxygen ion movement in the scale. However, the gradual growth in thickness and possibly cobalt content of the outer cobalt-rich peak could only be achieved by the outward diffusion of Co^{2+} ions. There is therefore strong evidence for scale growth by cation movement in the present case, supporting most of the previous evidence with the pure metals. The cobalt peak was constantly reformed, the cobalt and nickel profiles being determined partly by the relative diffusion rates of the cations and partly by thermodynamic factors. It also appears that there was a general outward diffusion of ions and that newly ionized metal did not pass right through the film. Although Co^{2+} ions diffuse about 100 times as fast in CoO as do Ni^{2+} ions in NiO, the closeness of the ionic radii suggests that there should not be any vast difference in their rate in any given composition of (Ni,Co)O. The present direct measurement of concentration profiles in growing scales has certain obvious advantages over marker experiments and tracer techniques, at least in the present system.

(3) Scales on the pure metals almost certainly grow in the same way, by cation movement[1].

(4) Since concentration profiles through single-layer and double-layer scales are very similar[15], this possibly suggests, but does not prove, that they grow by the same mechanism. This would involve cation movement outwards in the inner layer rather than oxygen gas diffusion inwards.

We thank Prof. T. K. Ross for providing facilities and for his advice, Mr. B. W. Lambert for his collaboration with the electron-probe microanalysis and the Science Research Council for the provision of a maintenance grant to one of us (J. M. F.).

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STRUCTURE OF THE QUASI-STELLAR RADIO SOURCE 3C 273 B

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IN this article we show that: (1) the radio variations[1] of 3*C* 273 *B* are consistent with its red shift being cosmological; (2) the variable component of the optical continuum[2] of 3*C* 273 *B* may be partly due to inverse Compton collisions between relativistic electrons and their own synchrotron radiation. In this case the optical variations would be correlated with the radio variations, though with a phase delay.

The problem involved in (1) is to reconcile (*a*) the upper limit on the size of the source derived from the period of the variations[3]; (*b*) the lower limit on the angular diameter of the source derived from the absence of self-absorption down to some particular frequency[4,5] (assuming the synchrotron mechanism and that the electrons are radiating incoherently); (*c*) the distance derived from the red shift of the optical counterpart of the radio source. This problem first arose with the quasi-stellar source *CTA* 102, which Sholomitsky[6] reported to vary with a period of about 100 days at 940 Mc/s. In this case the distance derived from the red shift of 1·037 (ref. 7) is ~1,500 times greater than the upper limit permitted by (*a*) and (*b*). We recently proposed[8] a model of *CTA* 102 which would resolve this discrepancy, but recognized that it required a rather improbable geometry and so was very implausible. Since then Maltby and Moffet[9] have reported that *CTA* 102 did not vary appreciably at 970 Mc/s over a 3-year period ending about 2 years before Sholomitsky's observations began. While it is possible that *CTA* 102 varies sporadically, the more likely inference is that Sholomitsky's observations are in error.

The same problem arises with the quasi-stellar source 3*C* 273 *B*, the flux density of which at 8,000 Mc/s was found by Dent[1] to have increased by about 40 per cent in 3 years. This source shows no self-absorption in its spectrum down to at least 200 Mc/s (ref. 10) (in fact the spectrum is flat down to this frequency). For a magnetic field strength of 10^{-3} gauss this implies a lower limit on the angular diameter of 0·03 sec (while the observed[11]

angular diameter is 0·5 sec). If the period of the radio variations is comparable with that of the optical variations, that is ~12 years, the size of the radio source cannot exceed 3 pc and its distance cannot exceed 20 Mpc. On the other hand, the red shift of 0·158 (ref. 12) implies a distance of 470 Mpc.

The discrepancy would be resolved if the varying part of the source had an angular diameter ~ 10^{-3} sec. We therefore propose[13]:

Model I. This model consists of a source of angular diameter 0·5 sec and (roughly constant) flux density ~25 flux units down to at least 200 Mc/s, in the centre of which is a varying source of angular diameter $1{\cdot}3 \times 10^{-3}$ sec (corresponding to a linear diameter of 3 pc), and at minimum a flux density ~2·5 flux units down to the frequency ν_a at which self-absorption sets in. Such self-absorption would not show up in the observed spectrum of this composite source. For a magnetic field H gauss in the central source, $\nu_a \sim 4{,}000\, H^{1/5}$ Mc/s. For simplicity we assume that the central source varies because of an increase in its relativistic electron flux, while its angular diameter and magnetic field are unchanged. If the total flux density of the source is doubled at high frequencies at maximum, the flux from the central source must increase by a factor ~ 10. Thus the frequency ν_b at which self-absorption sets in at maximum is given by $(\nu_b/\nu_a)^{2{\cdot}5} \sim 10$, so $\nu_b \sim 10{,}000\, H^{1/5}$ Mc/s.

We can now predict the time variations that would be observed at various frequencies. For simplicity we take the central source to vary sinusoidally at high frequencies. For $\nu > \nu_b$ no self-absorption occurs, so the observed variation would be sinusoidal. In the intermediate range $\nu_b > \nu > \nu_a$ the flux density would at first increase sinusoidally, but when it reaches the level at which it would be self-absorbed at that frequency the increase would be cut off, and the flux density would remain constant until it decreases again in the second half of the cycle. For $\nu < \nu_a$ the variations would be negligible.

A lower limit on H (the value of which has not yet been specified) can be obtained if we require that the energy density of relativistic particles shall not exceed the magnetic energy density. If only ~ 1 per cent of the total energy is in the electrons, we find that the field strength cannot be much less than 1 gauss. If $H = 1$ gauss, ν_a and ν_b have the values ~4,000 and ~10,000 Mc/s respectively, and the expected variations at 8,000 Mc/s are large enough to account for the observations. Our estimates for ν_a and ν_b are only rough, but they indicate what might be expected, and the measurement of their values and of the variations at intermediate frequencies would be a useful test of the model and would provide considerable information about the structure of the source.

It is not known how electrons attain relativistic energies in radio sources. The generation process may occur throughout the volume of the source, or the electrons may be ejected from a massive object at its centre. It is easily seen that the electrons must be produced by a mechanism of the former kind if the source in fact resembles Model I. The reason is that the lifetime of an electron which radiates at, say, 10^4 Mc/s in a field of 1 gauss is only ~5×10^6 sec, which is much less than the time taken to cross the source, even for an electron moving at the speed of light. If such an electron were ejected from the centre it would not reach the edge, unless it were emitting radiation at a frequency at which the source was opaque. Thus for frequencies exceeding ν_a our discussion of the variations will be incorrect. To allow for this we now introduce:

Model II. In this model we assume that the electrons are all generated in a very small region (with diameter < 0·1 pc), which presumably contains a massive object. As in Model I, nearly all the flux at frequencies < ~ 4,000 Mc/s is assumed to come from a halo, and we only concern ourselves with an intense spherically symmetric core with radius ~ 1 pc, in which the variable higher frequency radio flux is supposed to originate. We assume that $H \sim 1$ gauss throughout this region, and that its radio emission has a flux density $S(\nu)$ which may be variable. For each ν we can determine the minimum radius $R(\nu)$ of a sphere from which $S(\nu)$ could come, if it in fact comes from a region which is opaque at all frequencies up to ν. We find that $R(\nu) \sim 0{\cdot}3\ (S(\nu))^{1/2}(\nu/10^4)^{-1{\cdot}25}$ parsecs, when S is measured in flux units and ν in Mc/s. This radius can be compared with the distance $r(\nu)$ which an electron radiating at frequencies ~ν would travel in its lifetime, assuming that it moves with speed ~c. This is $\sim 5 \times 10^{-2}(\nu/10^4)^{-1/2}$ pc. If $R(\nu) > r(\nu)$, electrons moving outward from the centre cannot radiate freely without producing a radiation field the brightness temperature of which at frequency ν is higher than their kinetic temperature. Therefore they will conserve most of their energy until they reach a distance ~ $R(\nu)$ from the centre, where they can radiate freely.

The available data do not enable us to specify the flux density of the core precisely, but it is consistent with the observations to take $S(\nu)$ to be 10–30 flux units in the frequency range from 5,000 up to ~10^5 Mc/s. $R(\nu)$ is then several times greater than $r(\nu)$ throughout this range, and so we conclude that the radiation at frequency ν comes from the surface of a sphere of radius $R(\nu)$.

If electrons are ejected from the centre at a steady rate, $S(\nu)$ and $R(\nu)$ will adjust themselves to values which depend on the energy spectrum of the input electrons, and the flux of the source will be constant. If, however, the rate of input of electrons alters, $R(\nu)$ (and consequently $S(\nu)$) will change after the lapse of an interval of the order of the time for light signals to travel a distance $R(\nu)$. Thus changes in the behaviour of the massive object will cause changes in the radio flux. The maximum rate of increase of flux will be attained if $R(\nu)$ expands with speed ~c. An increase of $S(8 \times 10^3)$ from 2·5 to 25 flux units, which corresponds to an increase in $R(8 \times 10^3)$ from ~0·6 to ~1·9 pc, could therefore certainly occur rapidly enough to explain Dent's observations. We can also predict, on the basis of this model, that more rapid variations could occur at higher frequencies. Furthermore, if a sudden change in the rate of injection of particles produces changes in $S(\nu)$ at different frequencies, we would expect to observe the variations at higher frequencies before those at lower (even in the absence of intergalactic dispersion[14]).

We have seen that, if the core emits a significant amount of radiation at, say, 10^5 Mc/s, this radiation must come mainly from a sphere which is opaque up to that frequency. The total energy density of all the radio frequency radiation up to 10^5 Mc/s within it will be ~6×10^{-3} ergs/c.c. (compared with ~ 10^{-6} ergs/c.c. within Model I), and this very high value suggests that inverse Compton scattering might be important. The Compton lifetime of an electron of energy $\gamma m_0 c^2$ in this radiation field is $8 \times 10^9/\gamma$ sec. If $S(10^5)$ ~25 flux units, $R(10^5)$ ~0·1 pc and the time which an electron takes to drift out of this sphere is ~2×10^7 sec if its outward velocity ~ $c/2$. But the electrons radiating at 10^5 Mc/s have $\gamma \sim 300$, and so their Compton lifetime is of the same order. The most energetic photons would result from the scattering of 10^5 Mc/s photons by electrons with $\gamma \sim 300$, and their frequency would be[15] ~ 10^{10} Mc/s, which is in the ultra-violet range. Photons of lower energy (including the whole visible range) would be produced, and the spectrum of the scattered radiation would have a low-energy cut-off at a frequency depending on the smallest value of γ represented in the electron energy spectrum. The energy radiated by this process in the visible range may well be greater than that of the synchrotron radio emission. The exact relative importance of the Compton and synchrotron losses is very sensitive to the high-frequency cut-off in the radio spectrum (which we have taken as 10^5 Mc/s) to the value of H, to the precise position where the electrons are accelerated, and to their mean rate of outward drift. It would there-

fore clearly not be worth while to base an exact calculation on this crude model.

The foregoing rough arguments do, however, suggest that at least a part of the visible light emitted by 3*C* 273 *B* may have been produced by Compton scattering. Moreover, such a hypothesis would provide a natural explanation for the observed optical fluctuations of the source, since, if electrons are accelerated in the massive object in irregular bursts, there will be variations in the intensity of the scattered light. Nearly all the scattering occurs in a region of radius $\sim 0{\cdot}1$ parsecs (since not only the radiation energy density, but also the particle density, is much higher near the centre). Therefore, fluctuations with time-scales of the order of months, or even less may occur, and these are in fact observed[16]. The long period variations[2] ($\sim$ 12 years) in the optical luminosity may also arise from periodic variations in the rate of injection electrons, which, as we have already seen, can produce the observed variation at radio frequencies. This model therefore suggests that the radio and the long-period optical variations may be connected.

The increase in the radio flux observed by Dent coincided with a decrease in the optical luminosity, which had the most recent of its 12-yearly maxima in 1962. But according to this model one would expect periodic radio variations to lag (perhaps by several years) behind the variations at the centre which cause them, so this fact also accords with our model, and suggests that the radio variations, when they have been observed for longer, will also turn out to have a period ~ 12 years.

The visible light from 3*C* 273 *B* is unpolarized[17], whereas the radio flux (at $\sim 3{,}000$ Mc/s) is ~ 3 per cent polarized[18]. It will not be possible to find out whether the central part of the source is polarized at radio frequencies until the extent to which the degree of polarization changes when the flux varies has been examined. However, even if the radio flux from the core were polarized (implying large-scale uniformity in the direction of H), it would not follow that the scattered visible light must appear polarized, since it is mainly produced in an extremely opaque region, where the radio-frequency radiation will not be polarized even if the field is uniform.

It has been assumed in the foregoing that H does not change significantly when the density of the relativistic particles alters. This will be a good approximation if the magnetic energy is greater than the particle energy (a strength of 1 gauss is sufficient). The field is presumably 'anchored' to the massive object.

It should be emphasized that the Compton losses are significant in this model mainly because of the occurrence of synchrotron self-absorption, which prevents the electrons from radiating away their energy as fast as they otherwise would. Any electrons which are accelerated to high energies ($\gamma > 300$, say), and which therefore emit synchrotron radiation at frequencies which can escape freely from the source, will lose their energy in a time much shorter than the Compton lifetime, so they will contribute to the radiation at high radio frequencies ($> 10^5$ Mc/s), rather than in the far ultra-violet ($> 10^{10}$ Mc/s).

Model III. The first two models have taken no account of the fact that lines are present in the optical spectrum of 3*C* 273 *B*. According to Greenstein and Schmidt[19], these lines could be produced by gas of density $\sim 10^7$ particles/c.c., temperature $\sim 2 \times 10^4$ °K, and mass $\sim 10^5$ $M_\odot$. Though this gas need not radiate all the optical continuum, it must be incorporated in a complete model of the source. The model which we shall now describe indicates one way in which this can be done.

If the gas were in the form of a uniform spherical cloud, its diameter would be $\sim 0{\cdot}5$ pc, and it would be opaque to light of all frequencies because of scattering by free electrons. It would therefore be difficult to account for the observed fluctuations in optical luminosity with a time-scale of a few months, since if the variable flux were emitted by a small region within the cloud its variations would be smeared out and would not be observed. This difficulty would be eased if the density within the cloud were non-uniform, or if it had a filamentary structure[20], but there would remain the problem of feeding energy into the gas to balance its losses.

In an alternative configuration, suggested by Shklovsky[21], the gas is distributed in a thin spherical shell. We shall adopt this suggestion, and show that a model can be constructed on the basis of which the radio variations, and both the long and short period optical variations, can be explained.

If the radius of the shell is taken[21] as $4{\cdot}10^{18}$ cm, and its density as $\sim 10^7$ particles/c.c., its thickness will be $\sim 10^{17}$ cm if its total mass is $\sim 10^5$ $M_\odot$. The observed broadening of the spectral lines places an upper limit of $\sim 1{,}500$ km/sec on the velocity of the shell if it is expanding. If there is a mass of $\sim 10^9$ $M_\odot$ at its centre, a field of ~ 1 gauss would be strong enough to prevent the shell collapsing. Scattering by free electrons would be unimportant at all frequencies. The shell will be transparent to most photons of optical frequency, but will be opaque to radio waves at all frequencies below $\sim 10^5$ Mc/s because of free–free absorption. The observed radio flux must therefore originate outside the shell.

The gas in the shell would produce the observed line emission, and would radiate at a rate $\sim 10^{46}$ ergs/sec, mainly at visible wave-lengths. Its thermal energy content is insufficient to maintain this rate of energy loss for more than a few weeks, and so it must be absorbing energy at an equal rate from the interior. Since the shell absorbs radio waves, intense but unobserved radio radiation may exist in the spherical region within it, and this suggests the possibility that the shell may be absorbing enough energy in the form of radio waves to compensate for its losses in the visible range. This would require the production of $\sim 10^{46}$ ergs/sec at frequencies below $\sim 10^5$ Mc/s, which is ~ 30 times as great as the observed power radiated in this frequency range. However, such a high rate of energy production is not unreasonable since a sphere within which synchrotron self-absorption were taking place at all frequencies up to 10^5 Mc/s would need to have a radius of only $\sim 0{\cdot}8$ pc (little more than half the radius of the shell) to emit $\sim 10^{46}$ ergs/sec. Alternatively the required amount could be produced if the whole interior were opaque up to $\sim 7 \times 10^4$ Mc/s. The exact situation within the shell will depend on the energy spectrum of the electrons injected into it, but a sufficiently high rate of energy generation will be achieved if relativistic electrons with $\gamma < 300$ are produced at a rate $\sim 10^{46}$ ergs/sec. We must assume that there is a cut-off in the electron energy spectrum for $\gamma > 300$, since otherwise there would be intense radiation at frequencies above 10^5 Mc/s which would escape through the shell and produce observable radiation with a higher flux-density than is observed.

The observed radiation at frequencies below 10^5 Mc/s (apart from the component which comes from the halo) is, according to this model, emitted outside the shell by electrons which have escaped from the interior. An electron ejected from the massive object at the centre will be absorbing and emitting radiation at the same rate until it approaches the shell. Since the synchrotron lifetime of the electrons (in the absence of self-absorption) is of the order of the time which they take to pass through the shell, most electrons will lose their energy before reaching the exterior. Thus there radiation will not be observed directly, but will simply heat the gas. However, since the power of the observed radio radiation is much less than the power required to heat the gas, the observed radio flux would be produced even if only a few per cent of the electrons succeeded in escaping. Despite the high density of the gas in the shell, an electron passing through it has a 90 per cent chance of reaching the exterior without losing a significant amount of energy in ionization and

collisional losses. If the field ~ 1 gauss extends outside the shell, those electrons will radiate away their energy before they have travelled a further distance $\sim 0{\cdot}1$ pc unless self-absorption occurs outside the shell as well. Such self-absorption will occur at sufficiently low frequencies, though the situation is somewhat complicated by the fact that the electrons can radiate energy inwards into the shell.

The production of the visible light is more complicated. Much of it, both in spectral lines and in the continuum, comes from the gas in the shell. However, Compton scattering may also, as in Model II, produce radiation at optical frequencies. The radio radiation energy density in the volume enclosed by the shell is $\sim 2 \times 10^{-3}$ erg/c.c. if synchrotron self-absorption occurs at frequencies up to $\sim 7 \times 10^4$ Mc/s, and the corresponding Compton lifetime of an electron is $2 \times 10^{10}/\gamma$ sec. The highest energy electrons the synchrotron losses of which are balanced by self-absorption have $\gamma \sim 250$, and their Compton lifetimes are of the same order as the time they would take to travel outwards from the centre to the shell. The Compton losses, therefore, are significant, though their exact magnitude is highly sensitive to the precise model adopted. The frequencies of the scattered photons will, as in Model II, be mainly in the visible and near-ultra-violet, and most photons will escape through the shell, though those the frequencies of which correspond to intense spectral lines may be absorbed. Compton scattering of radio frequency radiation outside the shell will be insignificant.

We now consider how the observed variations in flux can occur in this model. We discuss first the long period variations. If there were fluctuations with a time-scale ~ 12 years in the rate of production of relativistic particles at the centre, there would be changes in the radiation density in the sphere enclosed by the shell, and a consequent change in the temperature of the gas. The rate of radiation of visible light by the shell would therefore alter. There would also be changes in the rate at which electrons escape through the shell, and consequently in the observed radio flux. Variations with periods less than, say, 5 years would be partly smeared out and so could not have a large amplitude.

The observed short period fluctuations in the optical luminosity have a time-scale of a few months, and obviously cannot be produced in the shell, which has a radius of $\sim$ 4 light years. In Model II they were attributed to the inverse Compton effect. Since we have shown that light will be produced by Compton scattering in this model, a similar explanation might also be possible here. This would require the scattering to occur in a region smaller than a few light months in radius. However, the scattering will not be concentrated in such a small region as in Model II, where the radiation energy density was high enough for scattering to be significant only within a sphere of radius $\sim 0{\cdot}1$ pc. Nevertheless, if all the electrons are ejected from a massive object, their number density will be higher near the centre, and the amount of scattering will be greater there even if the radiation density is no higher. Consequently, the variations in the component of the optical continuum produced by the Compton effect may be sufficiently rapid to account for the observed variations with time-scales of a few months.

We note that whereas the line strengths will alter with the slow period ~ 12 years, it would be inconsistent with this model for them to be involved in the rapid fluctuations in optical luminosity. We understand that attempts are now being made to see whether the lines do in fact vary in intensity. This feature of our model will thus soon be tested.

If we could construct a complete model of this kind, we would be able to calculate the expected phase lag between the variations in luminosity at optical frequencies and the related radio variations. However, we lack sufficient information to enable us to do this. To illustrate the complexity of the problem we enumerate some of the factors which would determine the magnitude of the lag.

(1) The variations in particle density and radiation energy density propagate outwards with different velocities.

(2) The phase delay of the radio variations will depend on whether self-absorption is taking place outside the shell at the frequency of observation. If it is taking place the observed flux would come from the surface of a sphere with radius greater than 1·5 pc, rather than from just outside the shell, thus increasing the lag of the radio variations. At 8,000 Mc/s, the frequency of Dent's observations, self-absorption would occur if more than ~ 14 flux units of the observed flux density were coming from the compact source (rather than from the halo). Since the observed variations amount to 10 flux units (and this flux cannot come from the halo), it is likely, though not certain, that self-absorption is in fact occurring.

(3) The sense in which the emission rate of the gas in the shell varies as the additional radio energy falls on it must be known.

(4) The relative intensity of the light produced by the inverse Compton effect and the light emitted by the shell must be known. These two components will have the same period, but may be out of phase with one another. The times at which maxima in the resultant optical luminosity occur will thus depend on the phase lag between the two components, and on the ratio of the amplitude of their variations.

This discussion shows that existing observations do not suffice to determine a well-defined working model of 3*C* 273 *B*. However, we can draw the important conclusion that neither the optical nor the radio variations require the source to be closer to us than the 470 Mpc implied by a cosmological red shift of 0·158. This conclusion considerably weakens the case for the local model[3,22] of quasi-stellar radio sources which places them a few megaparsecs away and in which the observed red shift implies a large velocity relative to their surroundings. More detailed observations of optical variations, and of radio variations as a function of frequency, should enable us to decide whether models of the type described here are appropriate for 3*C* 273 *B*, and for other quasi-stellar sources as well.

Note added in proof. H. Gent and H. P. Palmer reported at the Dublin meeting of the Royal Astronomical Society (September 7, 1965) that they have succeeded in resolving 3*C* 273 *B* at 21 cm with a long base-line interferometer. Their proposed interpretation of their observations is that the source consists of two components separated by $\sim 0{\cdot}4$ sec of arc, the angular diameter of each component being less than $\sim 0{\cdot}1$ sec. This limit on the angular size of the components is still much greater than the angular diameter of the variable core in our model ($\sim 10^{-3}$ sec), and of the lower limit of 3×10^{-2} sec for the halo.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Rotation Period of the Planet Mercury

THE recent radar measurements of Mercury indicate that the period of rotation of the planet is 59 ± 5 days[1]. This result is in complete disagreement with the previously quoted value of 88 days based on the visual observations of the markings on Mercury[2–6]. In this communication we show that the same visual observations can not only be reconciled with the radar-determined rotation period of Mercury but, in addition, can be used to derive an improved value for the period of rotation of the planet, namely, 58·4 ± 0·4 days.

We have examined nearly 50 drawings of Mercury published by Lowell[2], Antoniadi[3], Lyot[4], Dollfus[5], and Baum[6]. Of these, six pairs of drawings show near duplication of markings and phase.

	Time-interval T (days)
(1) Antoniadi (August 11, 1924) and Antoniadi (June 21, 1927)	1,105
(2) Antoniadi (August 23, 1927) and Antoniadi (August 6, 1928)	349
(3) Antoniadi (October 4, 1927) and Antoniadi (August 23, 1929)	689
(4) Antoniadi (August 6, 1928) and Antoniadi (July 20, 1929)	348
(5) Lyot (July 22, 1942) and Dollfus (October 12, 1950)	3,004
(6) Baum (March 15, 1952) and Baum (March 1, 1953)	351

Duplication of markings and phase for any single pair of drawings of Mercury does not necessarily indicate that synchronous rotation of the planet is the only possible solution. A number of other periods of rotation are possible as given by the following equation:

$$P \approx \frac{T}{n + \frac{\theta}{360}}$$

where P is the period of rotation of Mercury in days, T the time-interval between the two observations, n the number of complete rotations of the planet, and θ is the average angular displacement of the earth and Mercury in their orbits, with respect to the stars, in time T. Fig. 1 shows all possible values of P between 50 and 70 days as calculated for each pair of drawings. It is noted that in addition to an 88-day period (not shown in Fig. 1) there are at least three more values of P, namely, 50·1, 58·4 and 70·2 days which will be consistent with all the six pairs of drawings. However, only one of these values is within the allowed limits of radar results (59 ± 5 days). Therefore the rotation period of 58·4 ± 0·4 days is consistent with both the visual and the radar observations of Mercury.

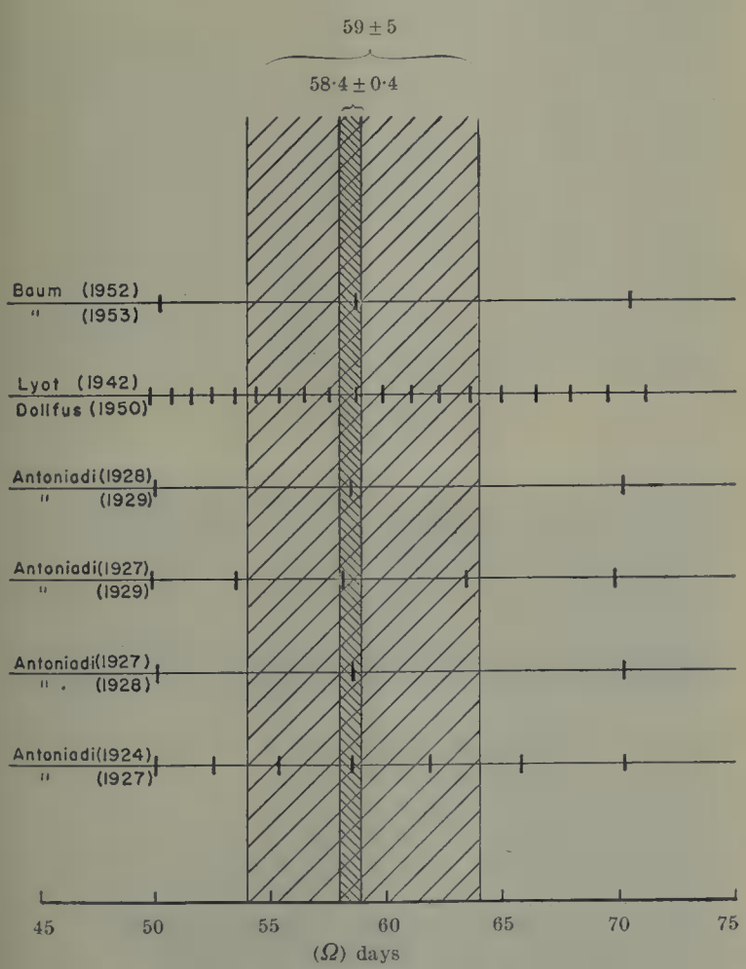

Fig. 1. Rotation periods of the planet Mercury, in days, as derived from six pairs of drawings. The single-hatched area shows the limits in the rotation period allowed by the radar observations of Mercury. Visual observations indicate a value of 58·4 ± 0·4 (double-hatched area)

Peale and Gold[7] have pointed out that because of the large eccentricity of the orbit of Mercury, the tidal torque will be greatest at the perihelion, and the planet will acquire a rotation period lying between 56·6 and 88 days. Our value of 58·4 ± 0·4 days is very close to the lower limit, indicating a significant amplitude dependence of the tidal dissipation on Mercury.

We thank Dr. A. G. W. Cameron for discussions.

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Tidal De-spin of Planets and Satellites

RECENTLY, Peale and Gold[1] have shown that the non-synchronous rotation of Mercury is likely to be a consequence of tidal friction. They point out that in an eccentric orbit the spin of an axially symmetric planet will not relax to the orbital mean motion, but instead will approach a final value which is somewhat larger. The final spin rate will be somewhere between the mean orbital angular velocity and the orbital angular velocity at perihelion. The precise value for the final spin is determined by the condition that the net tidal torque on the planet around each orbit be equal to zero. The spin rate at which this condition is satisfied is determined by the frequency and amplitude dependence of the planet's 'Q' ($1/Q$ is the specific dissipation function[2]). According to Peale and Gold: "The condition discussed here is based on the supposition

that the solar torque exerted on the tidal bulge exceeds that exerted on any permanent deformation from axial symmetry. In the converse case a period of 88 days for the rotation would indeed result." This last statement, if true, would imply that Mercury's principal moments of inertia, in the plane perpendicular to its spin axis, differ by less than a few parts in 10^7. This value is very small when compared with the values known for the Moon—another solid, slowly rotating body.

I have re-examined the conditions which determine which of the two final states of rotation tidal friction will bring about. While the conclusions of this investigation cannot be expressed in exact analytical form, good approximations do exist in the limiting case of low orbital eccentricity. These conclusions do not agree with those expressed by Peale and Gold in the statement already quoted here, except for the case of an axially symmetric planet.

As mentioned previously, the vanishing of the net torque on an axially symmetrical planet (the rotation and symmetry axes are assumed to be coincident with the normal to the planet's orbit plane) when averaged over an orbit leads to a final value for the spin, s, which is somewhat larger than the mean motion n. If the figure of the planet has a sufficiently small permanent deformation from axial symmetry, the only new feature which arises is an oscillation of s about its value for the axially symmetric case. Otherwise, if this deformation is large enough, s will eventually begin to oscillate about n and tidal friction will cause the amplitude of this oscillation to decay. Quantitatively, the division between a sufficiently small and a sufficiently large permanent deformation occurs at:

$$\frac{B-A}{C} \simeq \frac{2}{3}\left(\frac{s-n}{n}\right)^2 \qquad (1)$$

where s is the final value for the spin in the case of axial symmetry. In equation (1), C denotes the moment of inertia about the spin axes while A and B are the smaller and larger of the principal moments about axes perpendicular to the spin axis. For completeness, it should be mentioned that if the initial spin rate of a planet is smaller than its orbital mean motion (this includes retrograde spins) tidal friction may produce synchronous rotation unless the planet's tidal deformation is of the order of its permanent deformation from axial symmetry.

The Moon is unique in the solar system in that its spin has obviously been affected by tidal friction and the value of $(B-A)/C$ for its figure is known. Therefore, for the Moon we may ask the following question: If in its present orbit the Moon were given a much more rapid spin, would tidal friction once again bring the Moon into synchronous rotation? The answer to this question appears to be: No. To arrive at this result we must first calculate $(s-n)/n$ for the Moon. In the special circumstance that Q is independent of both frequency and amplitude, it is easily shown that $\frac{s-n}{n} \simeq 9{\cdot}5e^2$. Taking $\frac{B-A}{C} = 2{\cdot}05 \times 10^{-4}$ (ref. 3), we find that the right-hand side of equation (1) is 2·75 times as large as the left-hand side. Thus the Moon would end up spinning with a mean period about 2·86 per cent shorter than its present orbital period and would not return to synchronous rotation. This brings up the question of how the Moon's rotation became synchronous in the first place. To answer this question we would need to know both how the Moon's orbital eccentricity and its principal moments varied in the past. Of course, little can be deduced about the Moon's figure and we shall assume that it has not changed. On the other hand, it is possible to calculate the change in the Moon's orbital eccentricity due to tides. This calculation shows that tides raised by the Moon on the Earth act to increase the eccentricity while tides raised on the Moon tend to decrease it[4,5]. Without knowledge of the Moon's Q no definite conclusions can be drawn about the sign of de/dt. However, it seems likely that de/dt is positive and e was smaller in the past. This might explain why the Moon has a synchronous rotation since equation (1) would be satisfied for $e = 0{\cdot}043$, 22 per cent below its present value. Of course, once the Moon attained synchronous rotation this condition would be maintained even though the orbital eccentricity increased. Similar conclusions hold if the Moon's Q is dependent on frequency and amplitude so long as this dependence is not unusually severe.

Equation (1) allows us to derive an upper limit to $\frac{B-A}{C}$ for Mercury. The value we obtain (again assuming Q to be independent of amplitude and frequency) is 0·107 which is undoubtedly much larger than the actual value.

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PHYSICS

Laboratory Observations of Plasma Instabilities in a Dipole Magnetic Field

There have been a number of laboratory experimental investigations concerning the nature of plasma motion in a dipole magnetic field. Recently results have been reported concerning an investigation of a stable trapped plasma belt in the field of a small, permanent, uniformly magnetized sphere[1].

Further investigations using a much larger sphere indicate the existence of plasma instabilities. The experimental arrangement is quite simple. A permanently magnetized sphere, 6 in. in diameter, is placed in a suitably large vacuum chamber and made a negative electrode, while the wall of the chamber is made the positive electrode. A d.c. voltage of sufficient magnitude to break down the gas is then applied to the configuration.

When the gas breaks down, a number of plasma configurations occur. The first to be described is that of a stable, visible, plasma belt in the equatorial plane as shown in Fig. 1. As time progresses, an intense arc discharge occurs along a field line as shown in Fig. 2. When the arc occurs the visible belt disappears. The reason that the belt and two arcs appear in the photograph in Fig. 2 is because the picture is a time exposure. After a short time the arc is quenched and the stable belt re-appears. The duration of the stable configuration

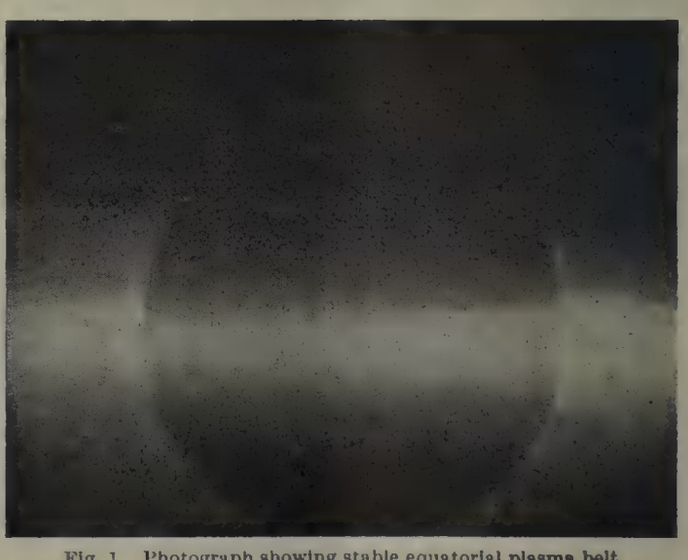

Fig. 1. Photograph showing stable equatorial plasma belt

increases with decreasing pressure and applied voltage. If the ambient pressure or the applied voltage is increased, both the stable belt and the arcs occur in a continuous manner. Such a condition is shown in Fig. 3.

The points of precipitation of the arcs also vary with applied potential. For large values of applied voltage the points all occur in equatorial regions as shown in Fig. 4.

Buneman[2] has discussed the various conditions under which instabilities in a streaming plasma can occur. Essentially the theory describes how the directed energy of an electron stream is dissipated in a plasma by collective collisions with ions. The theory treats both random fluctuations and fluctuations caused by the application of a constant field.

Swift[3] has applied the theory to a particular model in which the stream is the equatorial ring current and a local electric field transverse to the magnetic field causes a perturbation. The model is applied to geophysical conditions, and the subsequent analysis suggests that, with a large enough ring current, an accelerating potential can be established along a field line provided the conductivity in that direction is not infinite. This potential then serves as an accelerating mechanism for auroral electrons.

Present experimental results indicate that the laboratory model is a particular case of instability arising from the action of a constant transverse electric field. The visible observations in the photographs certainly indicate that a stable configuration first becomes unstable, then returns to its former configuration. Additional evidence is also found in the measurement of the temporal dependence of the instability by another method. A Langmuir probe was placed in the stable belt in the equatorial plane. The potential of the probe with respect to the sphere was measured as a function of time. A sketch of a typical oscilloscope trace of the event which is remarkably reproducible in almost all its details is shown in Fig. 5. Starting with the stable configuration, the voltage is seen to increase slowly with time until a critical value is reached. At this potential there is a sharp rise by at least a factor of 5 and a sharp decrease to some value above the stable case. This sharp rise and fall occur in the order of microseconds. There follows a constant voltage regime which lasts for approximately 100 msec during which the arc is sustained. Then the voltage drops to the stable value in approximately 50 msec. The whole process is then repeated. It appears that with proper variation of pressure and voltage the stability conditions can be varied over a wide range.

The present experimental evidence, while incomplete and only moderately quantitative, suggests that much can be learned from further, more extensive, investigations which will include a complete mapping of the temporal and spatial dependence of the instabilities by measurement of electric and magnetic field variations as well as transient current systems. Such investigations are at present in progress.

Fig. 2. Photograph showing arc instability. Two appear as a result of 5-sec exposure

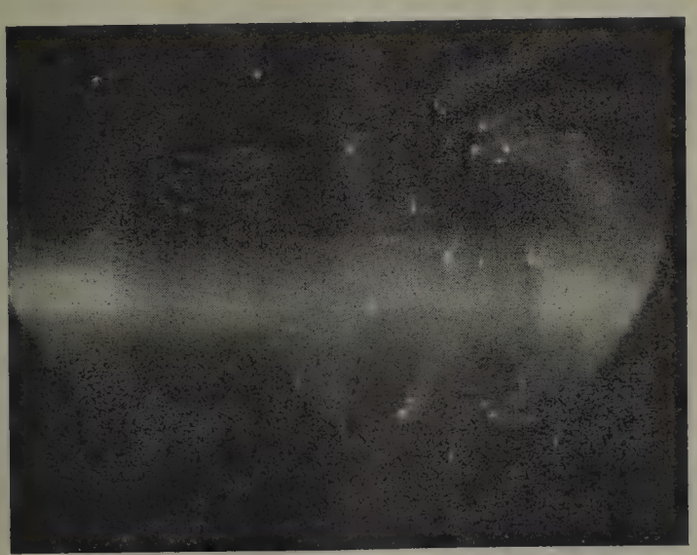

Fig. 3. Photograph showing more rapid occurrence of instabilities at higher pressure and voltage. (Darkened area is outline of viewport)

Fig. 4. Photograph showing constriction of precipitation points to low latitudes at high voltage. (Darkened area is outline of viewport)

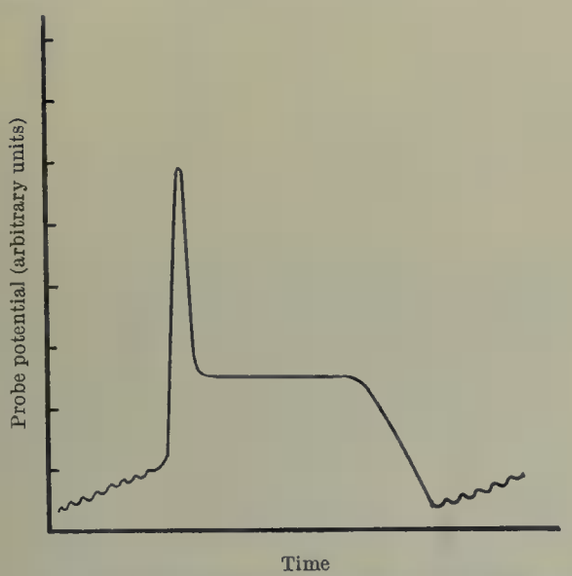

Fig. 5. Sketch of probe potential versus time. The sharp increase and decrease time-interval is of the order of microseconds. The constant voltage time-interval is approximately 100 msec, while the decrease to initial conditions lasts approximately 50 msec. The wavy portion which indicates the slow rise to instability is of the order of seconds

While the model experiments do not accurately reproduce geophysical conditions it is hoped that a careful experimental study will lead to the formulation of a theory which can then be extended to explain various aspects of auroral phenomena.

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Sub-millimetre Wave Spectra of Polar Liquids

In an earlier communication[1], it was reported that liquid phase absorption of the monohalogenated benzenes at a frequency $\approx 10^{12}$ c/s (1 teracycle) was higher than would be expected from the assumption that a simple Debye process was operative. The measurements supporting this conclusion were made using a *CN* maser source which gives only the single frequency 29·715 cm^{-1} (337μ wave-length). We have now supplemented these observations by wide-band spectral investigations using Fourier transform interferometric techniques.

The lower part of Fig. 1 shows results for chlorobenzene in the spectral range 10–400 cm^{-1}. The sharp absorption features at 297 cm^{-1} and 195 cm^{-1} are known molecular vibration bands ν_{24} (B_1) and ν_{30} (B_2), respectively, and are the lowest frequency modes of the molecule[2]. However, the main interest here is the region below 100 cm^{-1}, and in the upper part of Fig. 1 this region is plotted on a logarithmic frequency scale. The microwave observations are those of Poley[3] and that of Garg and Smyth[4], and the interferometric measurements confirm the earlier maser observation. It can be seen that there is a broad but strong feature reaching a maximum at a frequency of 45 cm^{-1} and that the peak is substantially greater than the asymptotic value to be expected at high frequencies from Debye's expression. These observations are believed to be the first reported of a complete band of this type though the high-frequency end of it is to be seen in the observations of Wyss *et al.*[5]. That such bands should exist is not surprising since they may be considered to arise from the

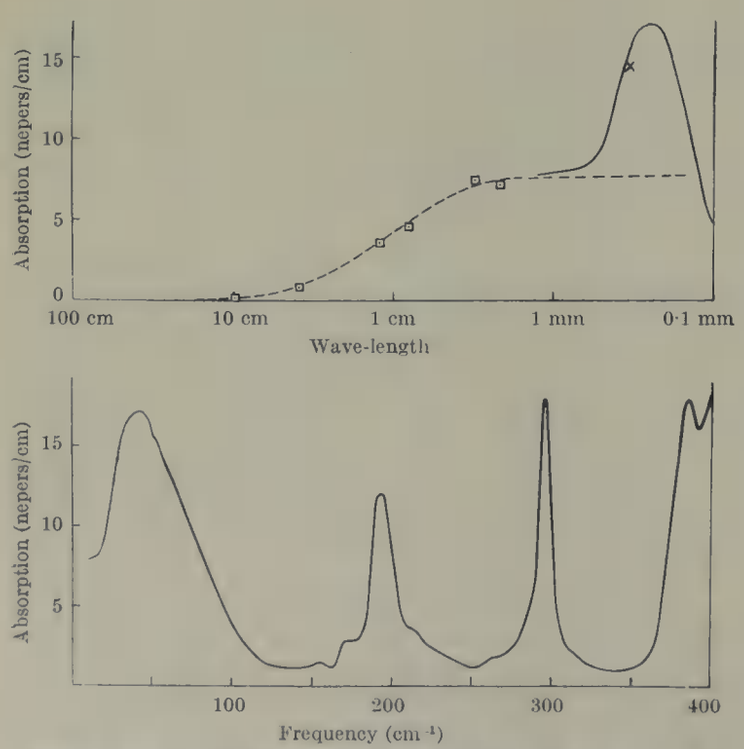
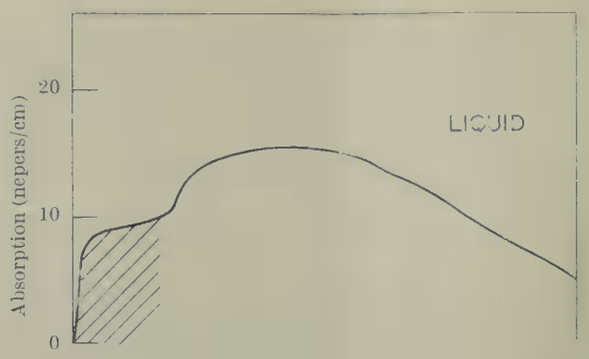


Fig. 1. Far infra-red spectra of liquid chlorobenzene. □, Microwave observations; ×, teratron observation; —, interferometer observations; – – –, Debye behaviour



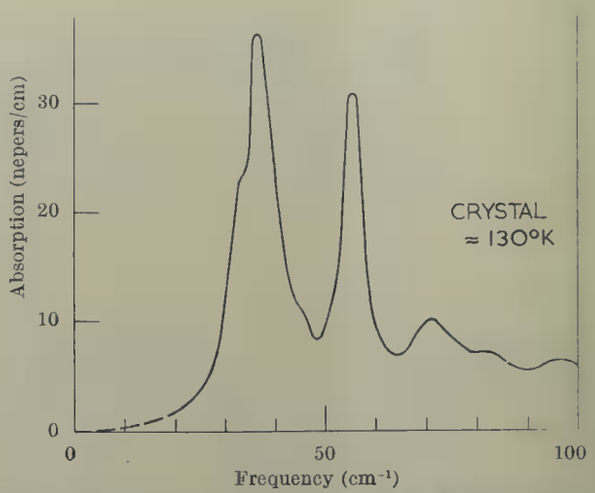


Fig. 2. Sub-millimetre absorption of chlorobenzene

'pseudo-lattice' modes of the liquid, and for this there is support from the observations shown in Fig. 2. This shows the absorption spectrum of solid chlorobenzene at a temperature of $\approx$130° K with again the liquid spectrum (at room temperature) for comparison. The solid spectrum shows the relatively sharp features characteristic of a lattice spectrum, and it will be seen that these occur in the same frequency region as the diffuse band observed for the liquid. It should also be noted that the absorption in the region below 20 cm^{-1} for the liquid (shown cross-hatched) where Debye processes are dominant is absent in the solid, as is expected since it arises from the overall rotation of dipoles.

The exact nature of the pseudo-lattice in such liquids will be clarified by the results of further work, but the general picture of a polar molecule undergoing resonant oscillations in the force field of its immediate neighbours is acceptable. In such a model, the width of any absorption line would be determined by the lifetime of the pseudo-lattice state or, in other words, the reciprocal of the time over which the surrounding cage does not alter appreciably. This latter is the relaxation time, which is known from viscosity measurements or more directly from the frequency at which the Debye asymptote is nearly achieved. This is about 10 cm^{-1} for chlorobenzene, and it is not hard to believe that the observed band is made up of a number of lines having widths of this order. The well-defined peak of the band is then explained by the reasonable assumption that the orientation of the oscillator in the 'cage' is not random, but has a most probable value which could be similar to that found in the crystal.

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METALLURGY

Effect of Temperature on the Discontinuity in the *S/N* Fatigue Curve of a Stainless Steel

RECENT investigations of the fatigue characteristics of a wide range of alloys have revealed a discontinuity in the *S/N* fatigue curves at high-stress levels. Finney[1] reports that the discontinuities in extruded high-strength aluminium alloys of Australian manufacture to specifications *DTD* 683/3 and 2024–*T*4 involve a shift to shorter fatigue lives at stresses above the discontinuities. These tests were carried out in rotating cantilever machines with notched test pieces ($K_t = 1{\cdot}5$), and Finney also showed that the size of the discontinuity increases with increasing rate of cyclic loading in tests within the range 100–1,200 c/min. A similar tendency was observed by Williams and Mitchell[2] in rotating cantilever tests of smooth test pieces prepared from weldable aluminium alloy—'Alcan 3032*WP*'. The discontinuities reported for tests on steels[3] show an opposite tendency to increased fatigue lives immediately above the discontinuities, and the trend is similar for both a ferritic steel to *BS*970*EN*3*B* and an austenitic stainless steel *S*130, when smooth test pieces are fatigued in a Rolls-Royce fatigue machine.

The strain ageing phenomenon in steels induces special characteristics in their fatigue behaviour, for example, the appearance of a 'knee' in their *S/N* curves[4]. It has been suggested that the discontinuity is associated with 'cyclic creep'[5] involving a general yielding of the metal due to exhaustion of strain hardening. The plastic deformation involved would induce hysteretic heating in steels and thus cause an increase in both dislocation density and diffusion rates to enhance strain ageing effects. To assess the role of strain ageing during the fatigue cycling of an 18/8 stainless steel, comparative fatigue tests were undertaken at room temperature and at −80° C. Calculations showed that the diffusion rate would be very slow at −80° C and strain ageing thus suppressed.

The tests were performed in a pull–pull fatigue machine utilizing flat specimens 0·061 in. thick and containing a drilled hole ($K_t = 2{\cdot}5$). Figs. 1 and 2 show the results

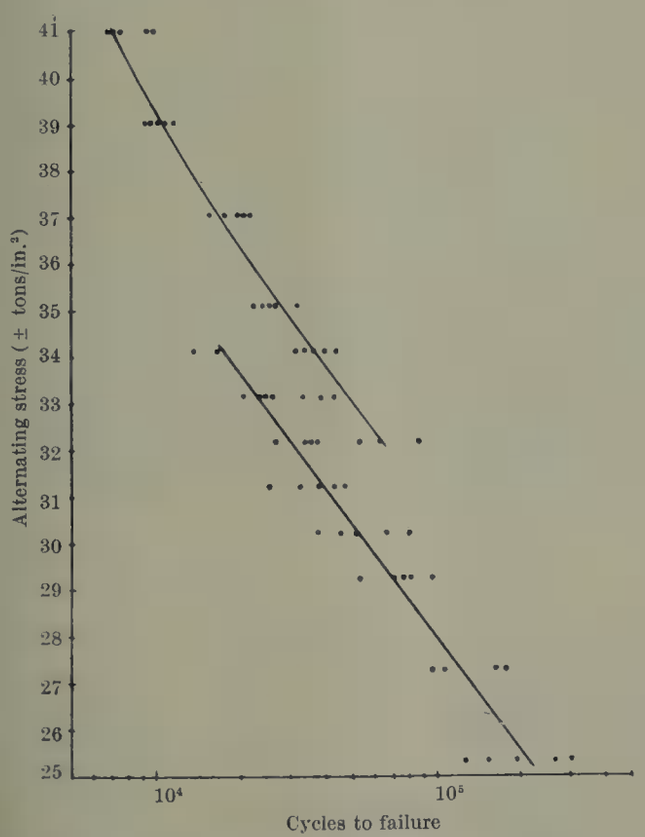

Fig. 1. Conditions: temperature, +20° C; loading, sinusoidal; frequency, 120 c/s.; stress concentration factor, 2·5

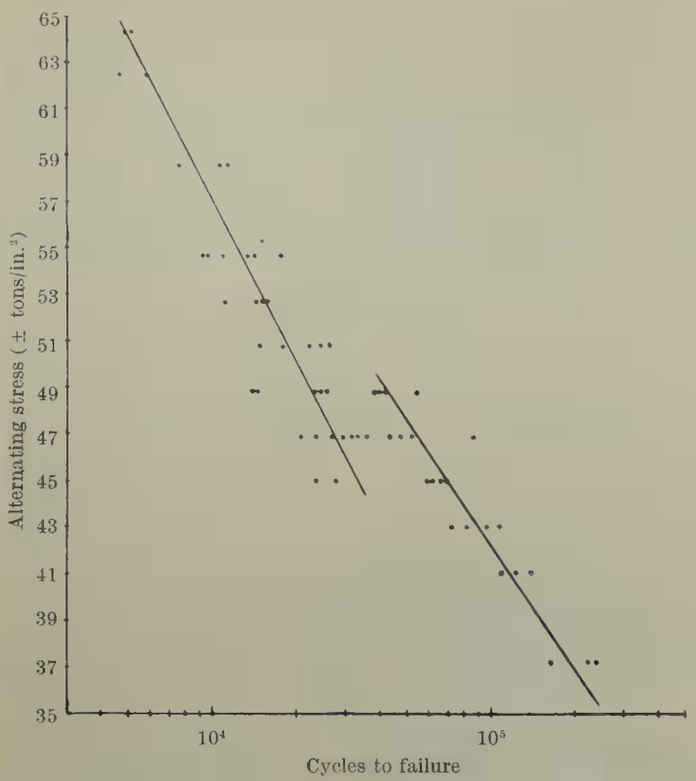

Fig. 2. Conditions: temperature, −80° C; loading, sinusoidal; frequency, 120 c/s; stress concentration factor ,2·5

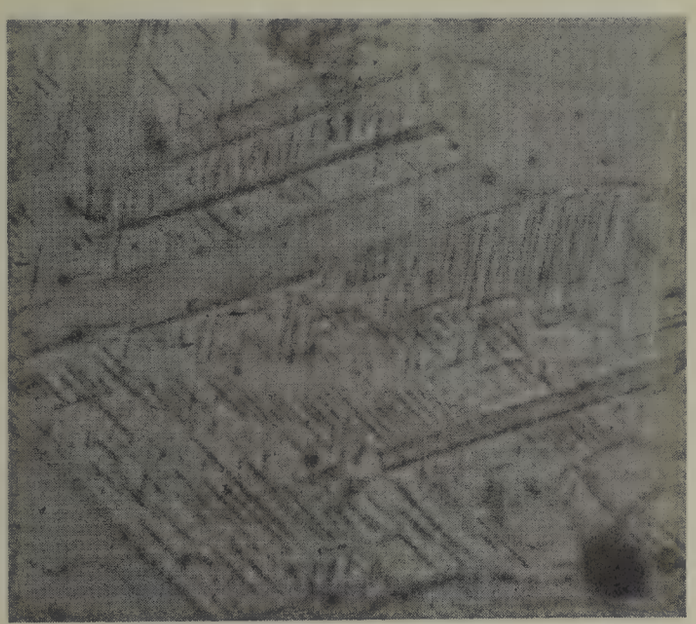

Fig. 3. (×*c.* 666)

obtained. Testing at −80° C has raised the stress-level of the discontinuity by about 15·9 tons per in.² and the shift of the curve above the discontinuity is to shorter fatigue lives. Metallographic examination of the fatigued stainless steel revealed extensive martensitic transformation at the slip bands (Fig. 3). It appears, therefore, that suppression of the strain ageing phenomenon is associated with a reduced resistance of the stainless steel to fatigue damage at stresses above the discontinuity.

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CHEMISTRY

Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential

In two earlier communications[1,2] it has been shown that the means of the first excited singlet state ^1L_b and first excited triplet state ^3L_a of some polycyclic aromatic hydrocarbons obey the equation:

$$I = 4{\cdot}39 + 1{\cdot}15\,\frac{^3L_a + {}^1L_b}{2} \quad \text{(eV)} \qquad (1)$$

where I denotes the ionization potential of the molecule. The designation of the states is due to Platt[3].

This equation is similar to the Hückel[4] equation:

$$I = \alpha + bE$$

where E is the energy of the first state and 'α' corresponds to the ionization potential of an infinite planar carbon molecule which can be assumed to be graphite, which has an ionization potential of 4·39 eV (ref. 5).

Hückel's theory does not consider electron spin and does not therefore differentiate between states of different spin multiplicity. It does seem that averaging out over the states of different multiplicity brings the experimental data into close agreement with the theoretical predictions.

The third excited states of the polycyclic aromatic hydrocarbons have been examined to see whether they show any correlation. The energies of higher excited triplet states are obtained by the techniques of flash-photolysis[6,7]. Values of T_3 are listed in Table 1. A plot of I against T_3 is shown in Fig. 1. A very rough correlation only is observed.

The energy of the third excited singlet state can be obtained from absorption spectra. The singlet state corresponding to the T_3 triplet state would appear to be the state designated ^1B_b by Platt. This in general corresponds to the level designed β by Clar[7]. In obtaining the values of ^1B_b shown in Table 1, the experimental values of Clar have been used throughout. In the case of phenanthrene and chrysene, theoretical studies by Ham and Ruedenberg[8] suggest that the levels designated β^1 by Clar correspond to ^1B_b and these have been used. The β^1 state has arbitrarily been designated ^1B_b for 1,2,5,6-dibenzanthracene, it having been found earlier[1] that these were a crossing-over of the first excited state. In all other cases the β state of Clar has been designated ^1B_b.

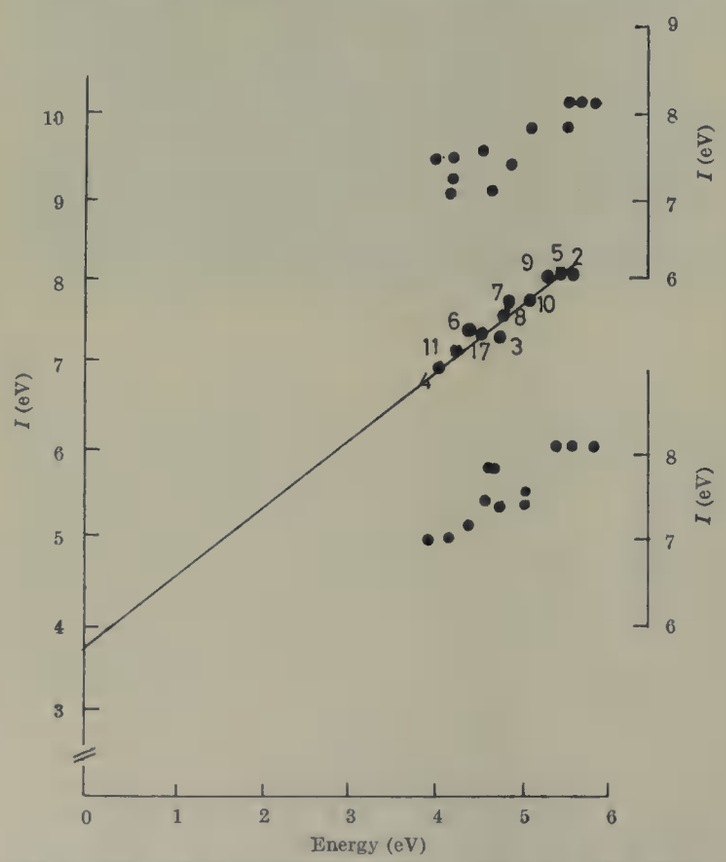

Fig. 1. Upper diagram: right-hand ordinate, plot of I vs. ^1B_b. Middle diagram: left-hand ordinate, plot of I vs. $\frac{^1B_b + T_3}{2}$. Lower diagram: right-hand ordinate, plot of I vs. T_3

A plot of I against ^1B_b is shown in Fig. 1, but only a rough correlation can be seen.

Fig. 1 also shows a plot of I against $\frac{^1B_b + T_3}{2}$. A fairly good correlation is seen to exist. The equation of the best straight line passing through the data is:

$$I = 3{\cdot}67 + 0{\cdot}82\,\frac{^1B_b + T_3}{2} \quad \text{(eV)} \qquad (2)$$

using the method of least squares. The sum of the standard deviations is 0·204 where $\frac{^1B_b + T_3}{2}$ is treated as the dependent variable.

The best straight line extrapolating to 4·39 is given by

$$I = 4{\cdot}39 + 0{\cdot}667\,\frac{^1B_b + T_3}{2} \quad \text{(eV)} \qquad (3)$$

The sum of the standard deviations is 0·315.

Hückel's theory predicts a correlation between I_2, the second ionization potential, and the third excited state, but not between I and this state. However, the capacitive energy model of Smith[9], which gives good predictions, states that there is a direct linear relationship between I and I_2 which will then explain the correlation shown in equation 2. The capacitive energy model predicts that:

$$I \simeq I_2 - 4{\cdot}39 - 3 \times 1{\cdot}5\,\frac{^3L_a + {}^1L_b}{2}$$

comparing equation 4 of ref. 9 with equation 1 of this report. This would therefore suggest that equation 3 is a more correct one to take, for, as the energy levels go to zero with increasing molecular dimensions, I should go to 4·39, the work function of graphite[5], the infinite carbon planar molecule. A good test of equations 2 and 3 is the prediction of the third triplet state of benzene. The ^1B_b state of benzene is 6·94 eV (refs. 7, 8). Equation 2 predicts that T_3 is 6·56 eV whereas equation 3 predicts that T_3 is 7·43 eV. It is hoped to build apparatus[10] which will enable the $T_1 - T_3$ transition of benzene to be measured.

For molecules having ionization potentials in the region of 7–8 eV, which includes nearly all twenty aromatic hydrocarbons listed in ref. 2, either equation can be applied. It is thus possible to predict the T_3 energies of those molecules for which it has not yet been observed. Alternatively, knowing T_3, the correct assignation of the ^1B_b singlet excited state can be made.

In Table 2 are shown the predicted energy-levels of some polycyclic aromatic hydrocarbons assuming ^1B_b equal to β and β^1. In all cases it is more probable that β^1 is ^1B_b, but values of β^1 are not available in all cases.

An interesting case is perylene. It has an ionization potential of 7·03 eV (ref. 2) and a T_3 energy of 4·10 eV (ref. 6). Equation 2 predicts a ^1B_b energy of 4·09 eV. The spectrum of perylene is greatly different from that of the

Table 1. Polycyclic Aromatic Hydrocarbons (Energies in eV)

		I (refs. 1, 2)	^1B_b (ref. 7)	T_3 (ref. 6)	$\frac{^1B_b + T_3}{2}$
(2)*	Naphthalene	8·10	5·60	5·62	5·61
(3)	Anthracene	7·37	4·92	4·74	4·83
(4)	Tetracene	7·00	4·22	3·96	4·09
(5)	Phenanthrene	8·09	5·65	5·26	5·46
(6)	1,2-Benzanthracene	7·45	4·34	4·60	4·47
(7)	Chrysene	7·80	5·12	4·62	4·87
(8)	Pyrene	7·55	4·55	5·05	4·80
(9)	Triphenylene	8·09	4·81	5·81	5·31
(10)	1,2,5,6-Dibenzanthracene	7·80	5·55	4·59	5·07
(11)	3,4-Benzpyrene	7·19	4·18	4·39	4·29
(18)	Coronene	7·44	4·05	5·05	4·55

* Numeration as in refs. 1 and 2.

Table 2

	I (ref. 2)	β (ref. 7)	β^1 (ref. 7)	$T_3^{(\beta)}$	$T_3^{(\beta^1)}$
1,2,7,8-Dibenzanthracene	7·68	4·07		5·72	
1,2,3,4-Dibenzanthracene	7·61	4·33	5·68	5·31	4·12
Picene	7·80	4·32		5·73	
1,2,4,5-Dibenzpyrene	7·27	4·04		4·74	
Anthanthrene	7·00	3·99	4·82	4·13	3·30
1,12-Benzperylene	7·35	4·08		4·90	
1,2-Benzpyrene	7·56	4·28	5·23	5·20	4·25

other aromatic hydrocarbons[6]. It has a new series of absorption bands denoted α^1 lying between the α- and β-levels. The energy of the α^1-level is 4·25 eV, which agrees reasonably well with the predicted value of ^1B_b. As this α^1-level is the third highest energy transition observed in perylene, the assignation to ^1B_b seems very sensible.

Equations 2 and 3 were computed by Miss E. Watson.

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Labelling of Beta-lipoprotein with Tritium

Labelling of organic compounds and bio-molecules with tritium is of great importance in biochemistry. In general, the method of choice may be radiochemical synthesis[1], the gas exposure labelling of Wilzbach[2–4], or biochemical synthesis or different types of exchange reactions[1]. However, for the labelling of certain bio-molecules in radiochemically pure form, without any destruction of the natural constitution, only biochemical synthesis could be expected to be successful.

In our laboratory β-lipoprotein was labelled with tritium by a simple exchange reaction in radiochemically pure form, without any change in its natural constitution. The method developed is a modification of that reported by Yavorsky and Gorin[5], and seems to be widely applicable for the labelling of different types of organic compounds and certain bio-molecules as well.

The tritiating reagent is $CH_3COOHT.BF_3^+$ CH_3COO^-. This complex can easily be prepared by refluxing a small excess of acetic anhydride with tritiated water and saturating the formed tritiated acetic acid with BF_3 gas. The complex was isolated by distillation (b.p. 139°–142° C).

Labelling of β-lipoprotein was effected by simply contacting and shaking it for 24 h with an acetic acid solution of the reagent in heterogeneous phase at room temperature. When the equilibrium was reached, the tritiated complex was decomposed by adding 3–5 ml. of water with subsequent lyophilization. The labile tritium was washed out with distilled water by repeated lyophilization. The labelled β-lipoprotein was used without any further purification.

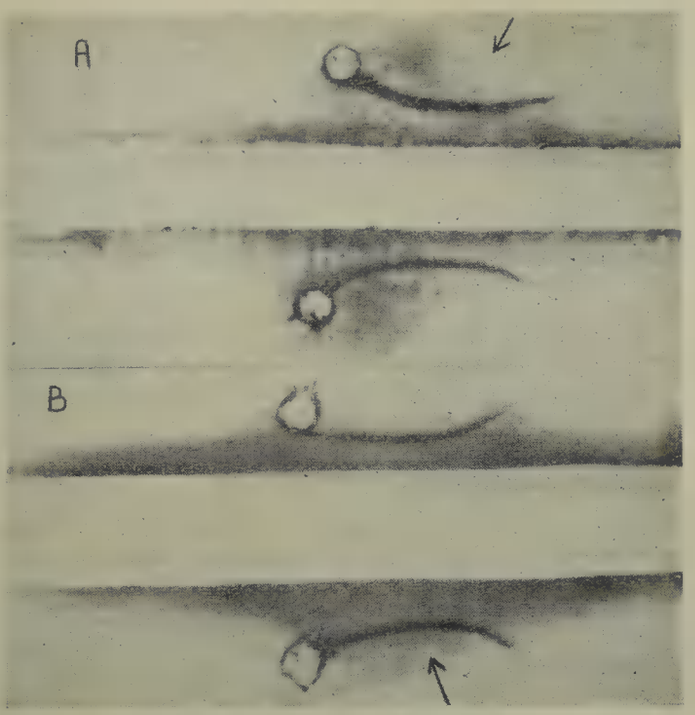

Fig. 1. Immunoelectrophoretic pattern of β-lipoprotein of rabbit. *A*, Above, after isolation by Gofman's method (arrow); below, the control β-lipoprotein used in our laboratory for identification; antisera, anti-rabbit β-lipoprotein produced in cockelers. *B*, Above, isolated β-lipoprotein before labelling; below, radio-immunoelectrophoretogram of isolated tritium labelled β-lipoprotein; antisera, the same as *A*

Similarly, some other compounds have also been labelled in order to get more detailed information about the scope and mechanism of this labelling method. Results are summarized in Table 1.

Natural β-lipoprotein was isolated by the method of Gofman[6,7]. The purity of the isolated sample was checked by the immunoelectrophoretic method of Scheidegger[8] (Fig. 1*A*). The same control proved (Fig. 1*B*) that during labelling the β-lipoprotein remained unchanged. The radiochemical purity of the labelled sample was checked by autoradiography using Kodak '*AR*-10' stripping film (Fig. 1*B*, lower picture). The labelled sample could be recovered from aorta homogenate in the same way[9–11] as unlabelled samples after having been administered intravenously into rabbits. The labelled β-lipoprotein has been used for some mechanistic investigation of plaque formation on cholesterol-fed rabbits. More details of this investigation will be published elsewhere[12].

Preliminary experiments made on different types of organic compounds showed that hydrogen atoms bonded to carbon can be replaced with tritium by this reagent (Table 1). The rate of these exchange reactions was found to be markedly dependent on temperature. However, the scope and particularly the mechanism of this exchange reaction require more detailed investigation.

The main advantages of this labelling method are: radiochemically pure labelled compounds can be prepared without any post-labelling purification that may be

Table 1. Equilibration of Different Types of Compounds with Tritiated Acetic Acid–Boron–Trifluoride Complex

Compounds	Weight (mg)	Reagents spec. act. (μc./mg)	Reaction				Products spec. act.	
			Weight (mg)	Time (h)	Temp. (°C)	Yields (%)	(μc./mg)	(μc./mmole)
β-lipoprotein	400	35·20	3,700	20	24	—	0·722*	
Phenylalanine	500	133·75	1,000	20	24	62	1·060	175·10
L-serine	500	133·75	1,000	20	24	90	0·877	92·16
L-thyrosine	500	133·75	1,000	20	24	46	0·914	165·61
L-tryptophan	500	133·75	1,000	20	24	30	2·523	515·25
L-methionine	500	133·75	1,000	20	24	63	—	
Cholesterol	500	131·28	1,000	20	24	45	3·023	1,168·81
Acetic acid†		133·75	1,000	2,000	24	—	114·08	6,845

* μc./ml. solution.
† Measured as the self-exchange of the complex.

required after other labelling methods, for example, after Wilzbach-labelling[4]. The procedure is rather rapid, requiring no special equipment, and may take place under mild conditions permitting the successful labelling even of sensitive molecules with suitably high specific activity. Activity yield is about of the same order as in the case of Wilzbach-labelling. Radioactivity was measured by the liquid scintillation technique using a Packard liquid scintillation spectrometer (model 3003).

We thank Dr. S. Virág for the isolation and purity control of the β-lipoprotein sample.

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Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate

The effects of irradiation and crystalline defect on pure quadrupole resonance (PQR) have been extensively investigated[1]. Randall *et al.*[2] observed an increase of the line width and second moment after X-ray irradiation, and concluded that strain broadening is the predominant mechanism. The changes in the electric field gradient induced by an applied stress were also measured by Collins and Bloembergen[3]. Colour centres occur in sodium chlorate crystals after γ-ray irradiation. These are probably due to V centres and O_2^- centres[4,5]. Such impurities are likely to set up stresses and bring about changes in the electric field gradient. For this reason, it would be expected that a shift of the pure quadrupole resonance would occur.

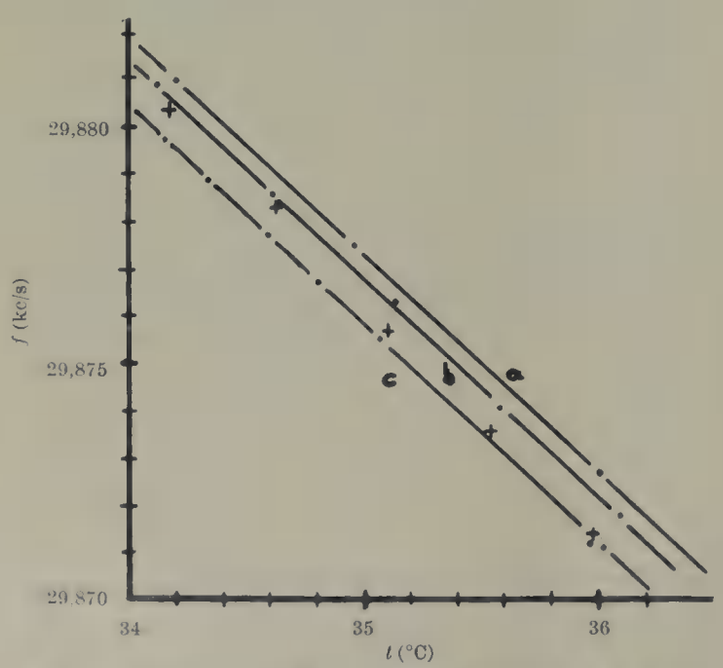

Fig. 1. Temperature dependence of PQR frequency of ^{35}Cl isotope: *a*, before; *b* and *c*, after γ-ray irradiations of $0{\cdot}9\times10^6$ and $2{\cdot}09\times10^6$ r. respectively

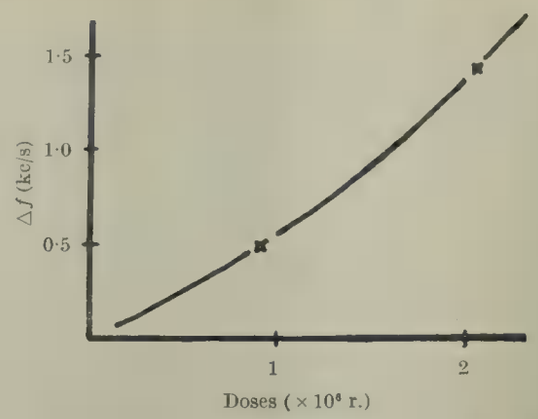

Fig. 2. PQR frequency shift of ^{35}Cl due to γ-ray irradiations

The aim of the work reported here was to measure this shift and to interpret the internal stress. The results of the preliminary measurements are shown in Fig. 1. It was necessary to eliminate the temperature effect. For that reason we made the measurements in a thermostat at about 35° C (ref. 6). In Fig. 1, *a* is for the non-irradiated sample, *b* is for the sample irradiated with γ-rays from reactor *TR*-1 (Çekmece Nüklear Research Center, Istanbul) with doses of $0{\cdot}9\times10^6$ r., and *c* gives the results after cumulative doses of $2{\cdot}09\times10^6$ r. During the measurements the temperature fluctuation was less than 0·01° C. Measurements were made with a conventional Dean-type spectrometer[7]. Frequency was measured with a Hewlett-Packard 524*C* period counter. For each point on Fig. 1 the mean square root error of frequency was about 30 c/s. In Fig. 2 PQR frequency shift is shown as a function of the irradiation dose. With these results and using theoretical analysis[3], it is possible to interpret the stress produced in sodium chlorate crystals by γ-rays. Since the piezo-electric effects are negligible[3], and since the changes in the electric field gradient may not explain the line broadening[2], the main effect should be due to strains. T^E_{3333} is very much greater than T^E_{3311}. The stress produced by rays of doses 10^6 r. in sodium chlorate crystals in the Na-Cl direction is:

$$\sigma_{33} \cong 23 \text{ kg/cm}^2\text{.Mr.}$$

I thank Dr. S. Akpinar, director of ÇNAEM, and Dr. A. Çilesiz for providing the irradiation facilities.

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Acid Phosphatases

We have been concerned with the isolation of acid phosphatases (orthophosphoric monoester phosphohydrolases, 3.1.3.2.) in quantities sufficient for chemical studies of these enzymes. The sources chosen were: the potato, the human prostate gland and human seminal plasma. We have found that the potato is a poor source and, although the enzyme has been obtained in a form which is homogeneous to starch-gel electrophoresis and to ultracentrifugation, the method is tedious and, because of the very large purification factor involved (about 25,000), leads to a low final yield (about 10 mg from 50 kg of potatoes). The best preparation previously reported was apparently associated with a purification factor of about 1,000 (ref. 1).

Several reports of the complete purification of prostatic acid phosphatase have been made[2,3], but, seemingly, the

amounts obtained were too small to allow the usual tests of homogeneity to be applied. It has also been reported that the pure enzyme is unstable[2] particularly so when in contact with glass surfaces. Recently it has been demonstrated by immunoelectrophoresis[4] that there are, in fact, two prostatic acid phosphatases. We now report that, by relatively simple and reproducible procedures, two acid phosphatases can be isolated from a homogenate of prostatic tissue; a typical preparation from thirteen glands giving 25 mg of P_2 and 10 mg of P_1. Both enzymes are homogeneous in the ultracentrifuge and on starch-gel electrophoresis and are unchanged on further attempts at purification. Both are stable and resist the following procedures: prolonged dialysis, ultra-filtration, gel-filtration, chromatography on cellulose ion-exchange resins, precipitation by salts, filtration through glass wool, prolonged periods (6 months) in solution at 4° interspersed with days at room temperature, low *p*H (about 2·0) freezing and thawing and contact with certain proteolytic enzymes. Both are unstable above 40° and on precipitation with acetone at 0°.

The assay method used for the enzymes is based on the fact that the ultra-violet absorption spectrum of a monoaryl phosphate differs from that of the parent phenol. For phenyl phosphate (*p*H 5·0, 0·1 M acetate) the difference in extinction coefficient at 276 mμ is 1,000. Assays are, therefore, conveniently carried out using a solution of phenyl phosphate (5×10^{-3} M) maintained at 25° in a spectrophotometer cell. The specific activity is defined as the rate of change of $O.D._{276}$/min produced when 10 μl. of enzyme solution is added to 2 ml. of substrate solution divided by the absorbancy of the enzyme solution at 280 mμ. This assay method is much more convenient and introduces fewer artefacts than aliquot methods based on the formation of molybdenum blue. Greater sensitivity can be achieved by using pyridoxal-5′-phosphate as substrate, but control of *p*H is then more important since the difference spectrum is very sensitive to *p*H changes.

Purification starts from an aqueous homogenate of minced tissue. The supernatant is fractionated by addition of ammonium sulphate; the enzymes precipitate between 50 and 75 per cent saturation. The fraction is freed from ammonium sulphate and brought to *p*H 5·2, 0·02 M acetate buffer. It is then absorbed into a column of DEAE-cellulose which has been equilibrated with the same buffer. After washing with dilute buffer, the column is washed with 0·2 M acetate buffer, *p*H 5·2. The active fraction is then concentrated by ultrafiltration and passed through 'Sephadex *G*-100' until its specific activity reaches 0·3. It is then re-chromatographed on DEAE-cellulose under the same conditions as described here except that gradient elution is used. The enzyme emerges as two separate peaks, P_1 coming before P_2. The specific activities are constant across the peaks and both have the value about 1·2 (this compares with the best value so far obtained with the potato enzyme of 0·4). The enzymes run at slightly different speeds on starch-gel electrophoresis, P_1 running slightly ahead. The Svedberg constants (20°, about 0·5 per cent protein concentration) are 5·23*S* and 5·36*S* for P_1 (3 determinations) and P_2 (6 determinations), respectively. Preliminary measurements of the diffusion constants indicate, assuming partial specific volumes of 0·745, molecular weights in the region 120,000–150,000. The rate of passage through 'Sephadex *G*-100', however, indicates rather lower molecular weights, that is, 110,000–130,000. Amino-acid analyses show some significant differences notably in the relative amounts of valine and alanine. With phenyl phosphate as substrate the two enzymes show similar kinetic behaviour. Both are inhibited by (+) tartrate. An important problem is whether both enzymes occur in each individual gland. In one experiment a single gland was found to contain both enzymes, but it is not yet known whether this is general.

Human seminal plasma might seem, at first sight, to be a convenient source of the prostatic enzymes. Accordingly, a sample of plasma, after dialysis, was chromatographed on DEAE-cellulose as described here. Starch-gel electrophoresis showed that the enzyme obtained, which had a higher specific activity than the prostatic enzymes (about 1·6), was free of contaminating protein. However, activity was found to be split into two bands and, consistently, in the ultracentrifuge the enzyme sample separated into two components, S_1 and S_2, with Svedberg constant (20°) of 2·65*S* and 4·43*S*, respectively. Afterwards a slight change in procedure separated the two enzymes on DEAE-cellulose. These enzymes clearly have much lower molecular weights than the enzymes isolated directly from the prostate and they may not be prostatic in origin. This problem is now being investigated.

The prostatic enzymes and the potato enzyme are inhibited by phosphate ions and by molybdate ions. In all cases the inhibition is kinetically strictly of the 'competitive' type. However, the inhibition by molybdate ions can be reversed by the addition of EDTA, whereas inhibition by phosphate ions is unaffected. The simplest explanation would seem to be that molybdate ions, unlike phosphate ions, combine with the enzyme in a way not directly involving the active site and produce a protein conformation unfavourable for combination with substrates. Consistently, the potato enzyme is considerably activated by EDTA and the effect can be shown not to be due to removal of trace metal inhibitors. These presumed conformational changes should, of course, be detectable by ORD measurements and the appropriate experiments are now in progress.

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Structure of Phosphobenzene

We have already shown that of the polyphosphines of formula $(C_6H_5P)_n$ and melting points (*A*) 149–150°, (*B*) 190°, (*C*) 252°–256°, (*D*) 260°–285°, compound *A* is a five-membered ring compound in the solid state[1].

Compound *B*, which has been prepared according to the directions given in the literature[2], crystallizes in at least four different forms; two of these, the monoclinic (from tetrahydrofuran) and rhombohedral (from benzene) modifications, have already been described[1]. The other two forms are triclinic and trigonal and have also been obtained by recrystallization from tetrahydrofuran. The triclinic form melted at 185°–189° in an open tube and 193°–198° in an evacuated tube, while the trigonal form melted at 190°–195° in an open tube and 236°–240° in an evacuated tube: all four forms decompose on melting. The only time the trigonal form was obtained the triclinic form was also present. It was not possible to define the conditions which would give any one of the three forms obtainable from tetrahydrofuran. The unit cell and space group data for the trigonal and triclinic forms are: Trigonal: a =

13·026, $c = 11·547$ Å, $D_m = 1·265$, $D_c = 1·269$ g/c.c., $Z = 2$, space group $P\bar{3}c1$. Triclinic: $a = 10·551$, $b = 14·717$, $c = 13·000$ Å, $\alpha = 73·7°$, $\beta = 63·2°$, $\gamma = 91·6°$, $D_m = 1·265$, $D_c = 1·266$ g/c.c., $Z = 2$, space group $P1$ or $P\bar{1}$.

We have determined the structure of the trigonal modification by single crystal X-ray diffraction methods. 879 of the 1,397 intensities measured with the aid of a Hilger and Watts linear diffractometer[3] were assigned a non-zero value and used in the structure determination. The position of the independent phosphorus atom, obtained from the three-dimensional Patterson function, showed that the six phosphorus atoms in the molecule were arranged in a puckered ring of the chair form. The positions of the light atoms were determined from the [c]-axis projection and a model. The structure was refined by the least squares method with individual anisotropic temperature factors. The final value of R for 879 planes was 0·072. Full details of the structure will be published elsewhere.

The analysis shows that, in the crystalline state, the trigonal form of phosphobenzene B is a hexamer, $(C_6H_5P)_6$, and contains a six-membered ring of phosphorus atoms in the chair form with phenyl groups occupying the equatorial positions (see Fig. 1). The molecule has the symmetry $\bar{3}$ ($C3_i$).

Although many attempts were made to prepare suitable single crystals of compound C, none was successful. In the only solvent found (diphenyl ether), the suspension had to be heated to refluxing point (258°) to get a clear yellow solution. On cooling only the pentamer, $(C_6H_5P)_5$, could be isolated. This conversion was most probably effected by heat alone, since it is known that, on melting compound C, compound A is obtained. It has now also been found that compound A is converted to C by heating in piperidine. Phosphobenzene C, m.p. 260°–265°, has been described as polymeric, but the value of n cannot be very large since an X-ray powder photograph shows it to be highly crystalline. Attempts to index this powder photograph have failed.

It is interesting to note that the related *cyclo*hexyl derivative, $(cyclo\text{-}C_6H_{11}P)_n$, is apparently tetrameric as suggested by previous workers[2,4]. It crystallizes in the space group $P4_2/n$ with $a = 15·33$ and $c = 5·63$ Å. The observed density is 1·161 g/c.c. which, with the cell volume, gives 924·2 for the molecular weight of the cell contents. This implies that there are two molecules of $(cyclo\text{-}C_6H_{11}P)_4$ (mol. wt., 456·5) in the unit cell with the molecular symmetry 4 (S_4). The sample used was supplied by Prof. Issleib of Halle.

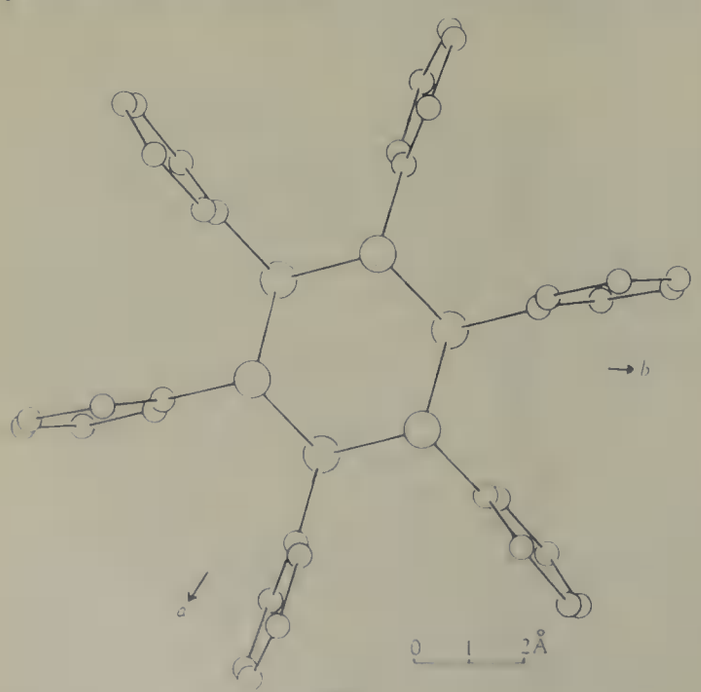
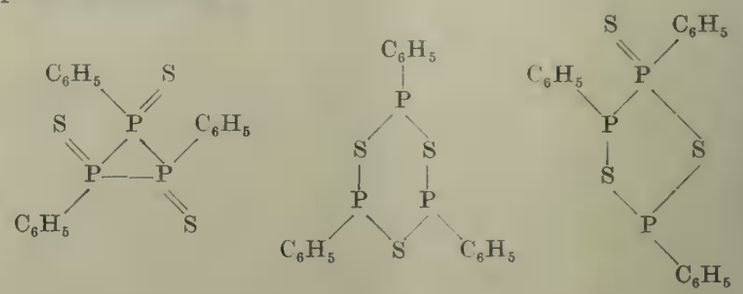


Fig. 1

Crystals of the sulphur derivative of $(C_6H_5P)_5$, $(C_6H_5PS)_n$, which were obtained from the reaction of phenylphosphine with sulphur (ref. 5) and were identical in every respect with those obtained from A and sulphur (ref. 6) (m.p. 148°, mixed m.p. 148°, superimposable infra-red spectra), crystallize in the monoclinic system. The space group is either $P2_1$ or $P2_1/m$, and a survey of the $h0l$ and $h1l$ intensities suggests that the non-centric $P2_1$ is more probable. The cell dimensions are $a = 8·90$, $b = 13·71$, $c = 8·35$ Å, $\beta = 107·5°$. The observed density is 1·438 g/c.c. which, with the known volume, gives six units (actually 6·005) of (C_6H_5PS) in the unit cell. Since in both possible space groups there must be a minimum of two molecules in the cell, it may be assumed that the molecule is a trimer. Some of the possible structures are:



It is not possible to evaluate a recent paper[7] describing the synthesis of co-ordination compounds obtained from transition metal carbonyls and what is apparently phosphobenzene A until full experimental details are published. Nevertheless the synthesis of $(C_6H_5P)_4Ni(CO)_3$ and $(C_6H_5P)_5Mo(CO)_5$ could be equally well explained by postulating a contraction of $(C_6H_5P)_5$ to give the nickel compound instead of an expansion of $(C_6H_5P)_4$ to give the molybdenum compound. We have made $(C_6H_5P)_5$ in two different ways[1] and have recrystallized it from three different solvents (benzene-alcohol, acetonitrile and tetrahydrofuran) and have always obtained crystals of the pentamer $(C_6H_5P)_5$. Thus it does not appear likely that acetonitrile has any special effect on phosphobenzene A as has been suggested by other workers[7].

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BIOPHYSICS

Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse

At the present time an increasing amount of attention is being directed to the non-collagenous proteins in connective tissues. Like collagen, these proteins are associated with polysaccharides in the form of mixtures or complexes, though the exact nature of the linkages between the moieties is a controversial subject. These compounds are essential components of the intercellular matrix of intervertebral disks, and a knowledge of them is needed for an understanding of the mechanisms of intervertebral disk prolapse.

Prolapse of intervertebral disk tissue in the lumbar region is a frequent cause of back pain and sciatica. Displacement of tissue follows after the loss of normal mechanical properties. It is known that in connective

tissues the collagenous fraction of the intercellular matrix is associated with tensile strength and the non-fibrillar fraction with resilience. The latter fraction requires further investigation.

The term 'herniation of the nucleus pulposus', though occasionally applicable, is an unfortunate misnomer. Disk prolapse is more correct, because the prolapsed tissue consists of varying proportions of nucleus pulposus and annulus fibrosus. Frequently large fragments of cartilage endplate are also to be found in sequestrated tissue, and it is misleading to compare the results of analyses on prolapsed tissue with similar investigations on the material excised from the centre of a normal control disk. The significant annular contribution to a disk prolapse is reflected in the high collagen content of prolapsed tissue reported by Davidson and Woodhall[1], and Mitchell, Hendry and Billewicz[2].

In a recent investigation on intervertebral disk tissue two main techniques were used, namely X-ray fibre diffraction and electron microscopy[3]. Subsidiary methods used were *in vitro* culture, histology, and biochemical analyses. A wide age range of fresh autopsy specimens (28th week of foetal life to 93 years) and tissue removed in the course of 56 lumbar laminectomies for prolapse was examined.

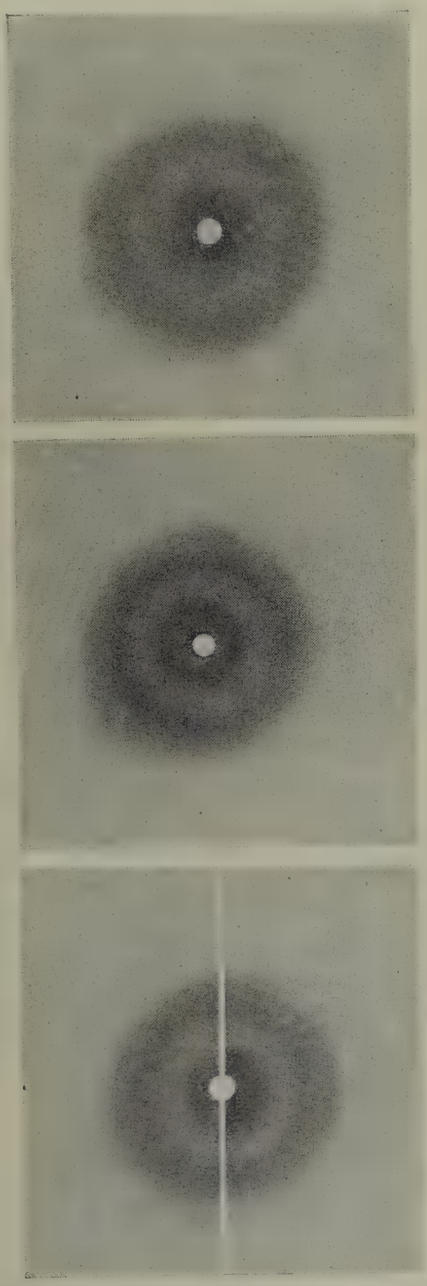
Little direct attention was paid to the polysaccharides since these are poorly crystalline and not suitable for exact study by the methods used. However, whenever the non-collagenous proteins are discussed the associated polysaccharides are implicated. In human intervertebral disk tissue, at least three non-collagenous proteins have been distinguished from each other by their X-ray fibre diffraction patterns and their appearances in the electron microscope. The differences are slight but quite distinct.

In young children the chondrocyte-like cell produces large amounts of a non-collagenous protein. This component has a 'fluffy' appearance when viewed in the electron microscope, indicating that there was a highly swollen gel structure before drying, and the 4·6 Å backbone spacing is a broadened reflexion (Fig. 1). The absence of a definite side-chain spacing is notable. By the middle of the second decade the cells produce what has been termed 'mature' β-protein. This is found in quantity in the nucleus pulposus and the transitional zones of the disk, and is present in comparable amounts at all spinal levels; it tends to become more crystalline with advancing age.

After the age of fifty years, and sometimes before, a third non-collagenous protein becomes a major component of disk tissue. In contrast to the proteins mentioned already it gives a better resolved backbone spacing and a well-defined side-chain spacing in the 10 Å region (Fig. 2). When observed in the electron microscope, it was seen to be relatively electron-dense. Since with advancing years this protein becomes a major tissue component, the physical properties of the tissue partly depend on it. Similar fibre diffraction patterns were not obtained in a study of normal articular and nasal septum cartilage, tendon or knee joint menisci[3]. The central fibro-cartilaginous area of the symphysis pubis from elderly subjects gives a comparable diffraction pattern, and in the electron microscope a similar dense protein has been noted in the media of the aorta. These observations suggest that the presence of this type of protein in certain connective tissues is connected with the demands of physical forces (the menisci are non-weight-bearing).

In sequestrated disk tissue there is a greater quantity of the mature type of non-collagenous protein as compared with tissue from the interior of the same disk; and in prolapsed disk tissue there is an increase in the non-collagenous protein content as compared with control tissue from the same age range. The tissue gives fibre diffraction patterns in which there is a prominent β-protein backbone spacing but the side-chain spacing is not present, indicating irregularity in the mode of packing of the polypeptide chains (Fig. 3). The chondrocyte-like cells were observed to be surrounded by large amounts of matrix, apparently of recent origin, suggesting that the increase of β-protein is a change associated with effect rather than cause. Moreover, the overall appearance of the tissue was consistent with a considerable reduction in quantity of the original matrix. The electron-dense protein was infrequently observed in disks where prolapse was present. A number of the specimens were from subjects in the age range where this component begins to be present in appreciable quantities.

Prolapse occurs most frequently in the age range 20–40 years and afterwards becomes decidedly less common[4]. This age distribution alone is sufficient to discount the hypothesis that prolapse is the result of pathologically accelerated ageing of the structure. Such a hypothesis



Figs. 1–3. X-ray fibre diffraction patterns copper $K\alpha$ radiation of wavelength 1·54 Å. Specimen to film distance ≈ 3 cm

Fig. 1. X-ray fibre diffraction pattern from the gelatinous nucleus pulposus of a child aged eight years. There is a broadened reflexion in the 4·6 Å region, but no definite side-chain spacing is resolved. The central scatter is from amorphous material. A weak reflexion at 2·86 Å is present from collagen

Fig. 2. X-ray fibre diffraction patterns from nuclear material of a subject aged 87 years. A well-defined β-protein diffraction pattern is present with clear resolution of the side-chain spacing. There is no evidence of orientation

Fig. 3. Comparison X-ray fibre diffraction patterns of prolapsed lumbar disk tissue (left) and nucleus pulposus from an elderly subject (right). The difference in the sharpness of the backbone spacings is evident. The non-collagenous protein in the prolapsed tissue has not given a side-chain spacing. (The region outside the collagen side-chain spacing is 'clean')

was tentatively supported by Blakely *et al.*[5], who also observed an increase in the β-protein content of prolapsed disk tissue, although they assumed that this was due to the same protein as is prominent in the disks of elderly subjects. Lyons *et al.*[6] reported the results of biochemical investigations during which they isolated an 'insoluble complex' from the disks of subjects over the age of 60 years taken as normal controls, and a 'similar type of complex' from eight 'herniated' specimens. Both complexes contained chondroitin sulphate *B*. These authors also supported the theory of pathologically accelerated ageing. The hexosamine/nitrogen ratios they reported for the insoluble complexes showed wide variations which could well be indicative either of a mixture or inconstant composition.

The changes in the intercellular matrix are, in the main, a reflexion of cell activity. In prolapse the polysaccharide content is reduced[1,2] and the non-collagenous protein content increased, an alteration in the quality as well as the quantity of the matrix. This indicates disordered cell function, which may well stem from a disturbed nutritional state similar to that which has been proposed as a cause of osteoarthritis. No differences have been detected between the tissue from patients with or without a history of a significant spinal injury. In ageing there is also a change in the type of matrix, with the emergence of the dense β-protein as a major matrix component. Once this is present in appreciable quantities it seems reasonable to suggest that it affords a measure of protection against prolapse.

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BIOCHEMISTRY

Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians

Melatonin (*N*-acetyl-5-methoxytryptamine) has been shown to be the most effective skin-lightening agent in amphibians[1,2]. This compound produces changes in pigmentation by causing the aggregation of melanin granules within the amphibian melanocyte. Although melatonin has been found to occur in mammals, no evidence for its formation has been obtained in amphibians where it exerts its most potent effects. High concentrations of radioactivity have been observed in the pineal area of the amphibian *Xenopus laevis* after the administration of the melatonin precursors ^{14}C-5-hydroxytryptamine and ^{14}C-methylmethionine[3].

The formation of melatonin is catalysed by an enzyme, hydroxyindole-*O*-methyl transferase, which requires *N*-acetylserotonin as the substrate and *S*-adenosylmethionine as the methyl donor[4]. So far, this enzyme has been found only in the pineal gland of mammals[5] and birds[6]. In mammals melatonin has been shown to inhibit the oestrous phase of the rat oestrous cycle and to produce decreases in ovary weight[7]. This communication describes the presence of hydroxyindole-*O*-methyl transferase in two amphibian species, *Xenopus laevis* larvae and adult frogs (*Rana pipiens*).

Table 1

Species	Hydroxyindole-*O*-methyl transferase activities
Xenopus laevis larvae	1·3
Rana pipiens adults:	
Brain	11·6
Pineal area	36·6
Optic tectum	34·5
Hypothalamus	36·0

Results expressed as mμmoles/^{14}C-melatonin formed per g tissue per h when incubated with *N*-acetylserotonin and ^{14}C-methyl-*S*-adenosylmethionine.

Xenopus larvae, stages 48–50 (ref. 8), were homogenized in 0·5 ml. ice-cold water using an all-glass homogenizer. A 0·2-ml. aliquot was taken to measure the hydroxyindole-*O*-methyl transferase activity[9]. A control incubation was run concurrently without the addition of substrate to correct for a small amount of endogenous material present in the *Xenopus* larvae which forms a methylated metabolite. On incubation of *Xenopus* larvae with *N*-acetylserotonin and ^{14}C-methyl-*S*-adenosylmethionine, a radioactive product was formed which was extractable into chloroform. This material had the same R_F value on three thin-layer chromatographic systems as authentic melatonin. The amount of melatonin formed enzymatically (Table 1) was more than enough to cause blanching in the *Xenopus*[2].

Hydroxyindole-*O*-methyl transferase was also examined in the adult frog brain using the procedure already described. Considerable enzyme activity was found in the frog brain (Table 1). Since the brain would also include the pineal, hydroxyindole-*O*-methyl transferase was measured in several brain areas. All areas examined contained enzyme activity. The melatonin-forming enzyme was found to be absent in the adult frog skin, liver, intestine and heart. These results demonstrate that the enzyme that forms the amphibian skin-lightening agent, melatonin, is present in the brain and pineal area of the frog, while it is present only in the pineal gland of all other mammalian and bird species so far reported. Later discoveries and more detailed analyses of hydroxy-*O*-methyl transferase activities in pineals, brain regions and eyes in various vertebrate species will be published elsewhere.

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Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats

A number of reports have recently been published dealing with the electrophoretic properties of water-soluble lens proteins of albino rats and the presence of a 'fast' anodic fraction in electrophoretic patterns of lens proteins from galactose- and lactose-fed rats[1,2]. It is well established that dense cataractous opacities develop in rat lenses within two to three weeks of galactose feeding[3]. Electrophoretic analysis of such lens extracts shows that the fraction with the highest anodic mobility is con-

siderably increased when compared to normal rat lens[4]. There is, furthermore, an overall decrease in fractions migrating toward the cathode.

Occasionally, however, a rat will not develop a severe opacity of the lens despite prolonged feeding with large amounts of galactose. In some instances the typical cataractous opacity is present in one eye only, while the second lens remains clear.

In an attempt to correlate the appearance of the 'fast' anodic fraction with the stage of cataract development, the following experiments were carried out. From a colony of approximately 100 male Wistar rats, which had been placed on a 40 per cent galactose diet, the following two animals were chosen at random from their respective groups: one rat from the group with mature bilateral cataract after 21 days of galactose feeding, and one rat from the group that had developed only a unilateral cataract. The rat from this second group had been on the galactose diet for 14 days during which time a nuclear opacity developed in one lens while the fellow eye remained clear. One rat of the same strain and comparable age, which had been fed normal laboratory rat chow, was selected as control animal.

After killing the rats under light ether anaesthesia, the lenses were immediately extracted from the eyes under a dissecting microscope and washed free of vitreous humour in iced physiological saline. Along with the dissection a schematic drawing was made of each lens to indicate the degree of cataract development. The lenses were then homogenized individually in 50 μl. iced saline in a small porcelain weighing-spoon, using a glass rod as pestle. The homogenate was transferred quantitatively into a micro-haematocrit capillary tube, using an additional 50 μl. of saline to rinse the spoon. The haematocrit tube was then sealed and centrifuged in a micro-haematocrit centrifuge for 4 min at 4° C. Following centrifugation, the supernatant was used for electrophoresis.

Agar-gel electrophoresis was carried out following Grabar's method[5], in a medium of 2 per cent agar in 0·025 M barbital buffer, *p*H 8·9, and 4 m.amp per cm for 55 min at room temperature. Immediately upon completion of electrophoresis, the agar slides were transferred into a staining bath of 0·05 M thiazine red in 5 per cent acetic acid. After staining for 1 h, excess dye was removed by washing in 5 per cent acetic acid with the final rinse containing 2 per cent glycerol in 5 per cent acetic acid.

Fig. 1 shows the results of the electrophoresis. The top two patterns represent lens protein extracts from the normal control rat, right and left lens being indicated at the cathodic side of the slide by *R* and *L*, respectively. The drawings next to the electrophoretic patterns indicate the appearance of the lenses under the microscope. As expected, the normal lenses were absolutely clear.

The next two patterns are those obtained from the lens extracts of the rat with mature bilateral cataract. These patterns correspond exactly to those which we obtained repeatedly when using pooled lens extracts from galactose-cataractous rats. The drawings show the presence of a dense opacity in the centre of the lens with small vacuoles in the periphery. Electrophoresis revealed a decrease in the cathodic fractions as well as a considerable increase in the fraction nearest the anode.

The third set of patterns stems from the lens extracts of the rat which had developed unilateral cataract after 14 days on galactose. The dense opacity in the left eye is smaller than that of the other rat due to the shorter feeding time. However, though the left lens still contains a normal amount of the cathodic fractions, the change in anodic fractions is already present. The right lens of this animal was perfectly clear except for a few isolated vacuoles along the periphery. The electrophoretic pattern of this lens is normal in appearance.

A sample of normal rat serum was electrophoresed along with the lens extracts to provide a reference marker. The serum protein pattern, indicated by the letter *S*, appears at the bottom of Fig. 1, the albumin showing at the right-hand side.

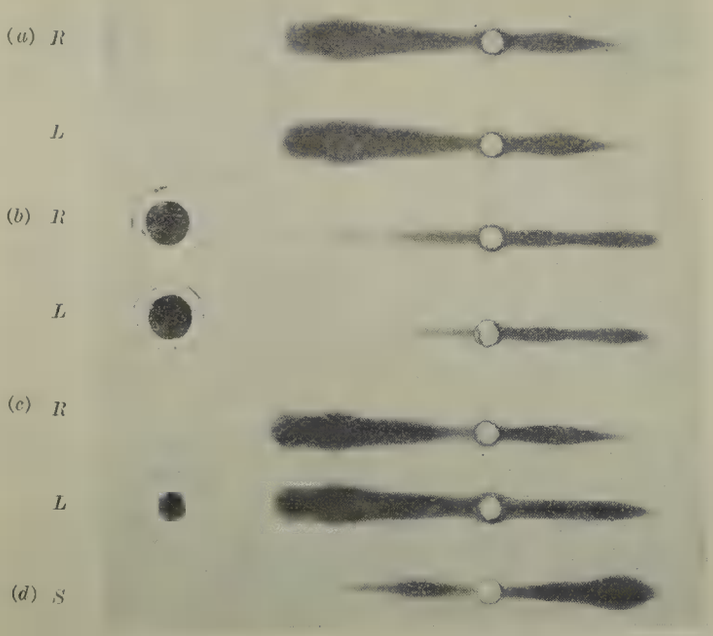

Fig. 1. Agar-gel electrophoresis of individual rat lens proteins. Right and left lens of the same animal are indicated by *R* and *L*, respectively. Drawings next to lens protein patterns at cathodic side show stage of cataract development as appearing under dissecting microscope at time of lens extraction. Anode at right. From top to bottom: (*a*) Normal rat; (*b*) rat with mature cataract after 21 days on galactose; (*c*) rat with unilateral cataract (left lens) after 14 days on galactose; (*d*) normal rat serum

Throughout our experiments, 38 out of 635 galactose-fed male Wistar rats, or roughly 6 per cent, did not develop bilateral cataract, even when feeding with galactose was continued for 4 weeks. The development of dense nuclear opacities in those rats was inhibited either in both eyes or in one eye only. There was, furthermore, no consistency as to which eye was affected when unilateral cataract was present. When the extracted clear lenses were examined under the dissecting microscope, the apparently unaffected lens showed the formation of vacuoles similar to those presented in Fig. 1.

In this work, the development of a unilateral cataract made it possible to use the clear fellow lens as a control, thus eliminating differences between animals. It raises the question whether a protective mechanism could be involved, lessening the effect of galactose on the metabolism of the rat lens. Why this should occur in one lens and not in the other is even more puzzling.

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Freeing of Amino-acids from Proteins

The large amount of free amino-acid which surrounds haemoglobin freshly released from red blood cells cannot be dialysed away from the protein even after vigorous washing (48 h, running water). The amino-acids still released after such washing may come from (*a*) metabolism of bacterial contaminants, (*b*) cellular enzymes,

Table 1

Time from completion of autoclaving (min)		Amino-acid released into dialysate from: Bag *A*	Bag *B*	Time from completion of autoclaving (h)		Amino-acid released into dialysate from: Bag *C*	Bag *D*
0	10	Cooling		0	1/6	Cooling	
10	15	3	4	These bags dialysed against running water from the conclusion of cooling until:			
15	20	2	6				
20	25	2	3				
25	30	4	3				
30	60	4	4	18	18·5	4	4
60	90	4	6	18·5	19	2	4
90	120	6	9	19	19·5	3	2
120	150	3	5	19·5	20	2	3
150	180	3	25*	20	20·5	3	23*
180	210	5	33*	20·5	21	4	17*
210	240	3	29*	21	21·5	6	10*
240	270	3	24*	21·5	22	8	25*
13·5 h overnight		9	42*				

Amino-acids expressed as μg of nitrogen in 100 ml. of dialysate.
* These dialysed against normal saline.

(*c*) amino-acids secondarily bonded to the protein, and (*d*) the cleavage of peptide bonds. It is impossible to rule out (*a*) and (*b*) in the case of haemoglobin.

Commercial casein, however, makes solutions with water which will withstand autoclaving (15 lb. per sq. in. for 30 min) without coming out of solution. A solution of casein subjected to this treatment does not have a large pool of amino-acids around it, but it does progressively release amino-acids on dialysis. (20 g of soluble casein (B.D.H.) were slowly added to 400 ml. of water at 30° C. 25 ml. of this solution contained 150 mg of nitrogen and was put into 'Visking' tubing double knotted at each end. These bags were immersed in water and autoclaved. They were dialysed against 100 ml. of liquid. Each dialysate was evaporated to dryness (1 g sodium chloride was added to the aqueous dialysates first, in order to standardize extraction). After extraction into acetone–hydrochloric acid mixture, which was removed at 120° C, the amino-acids were estimated by the method of Moore and Stein[1].) The autoclaving must exclude those having been produced by causes (*a*) or (*b*)[2].

The amount of amino-acid produced is always small, but it increases significantly if the dialysis is against normal saline instead of water. This is shown in Table 1, as is also the continual production of free amino-acids. The quantity released is in some way related to the concentration of amino-acid in the surrounding solution since the amount freed after 15 h dialysis against 100 ml. of unchanged water is no greater than the sum of the amounts given off during four successive half-hour dialyses.

The freeing of more amino-acids by salt was first noticed with solutions of haemoglobin. While in the present report on casein the cause (*c*) cannot be excluded, the evidence is in favour of the rupture of primary bonds.

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Red Pigment in Leaf Galls of *Salix fragilis* L.

The galls of *Salix fragilis* L. leaves are produced by *Pontania proxima* Lep. and contain a red pigment which is not present in mature leaves or leaves freed from galls, but has been found in young leaves[1]. Pigment production has been stimulated in young willow leaves by slight mechanical injury, but the injured leaves generally died[2]. Pontanin was isolated as orange-yellow needles from the galls of *S. fragilis* dried at 100° C and on hydrolysis gave 1 mol. of purpurogallin and 2 mol. of glucose, the probable formula being $C_{23}H_{28}O_{15}$ (ref. 3). Related glycosides of purpurogallin were isolated from other dried galls[3], but it was uncertain whether these compounds were natural products or artefacts, and from unpublished work[4] it was evident that fresh galls from one of the species used lacked purpurogallin glycosides. Hence the present work was done with a fresh approach to clarify the identity of the pigment as part of a larger investigation.

The pigment was extracted from the fresh galls of *S. fragilis* leaves by maceration with methanol containing 1 per cent hydrochloric acid, the extract concentrated to a few ml. under reduced pressure, and purified by the method of Harborne[5] on washed sheets of Whatman No. 3*MM* paper by band chromatography, using *n*-butanol–2 N hydrochloric acid (1 : 1 v/v, upper phase). The pigment band was then eluted with methanol–acetic acid (95 : 5 v/v), concentrated to a few ml. under reduced pressure and further purified by band chromatography using, first, *n*-butanol–acetic acid–water (4 : 1 : 5 v/v, upper phase), and, secondly, water–acetic acid (85 : 15 v/v). The pigment was eluted as before.

The purified pigment was evaporated to dryness *in vacuo*, re-dissolved in 2 N hydrochloric acid, heated in a boiling water bath for 20 min, then cooled and extracted with iso-amyl alcohol. Purification was achieved by band chromatography on washed sheets of Whatman No. 3 *MM* paper, using water–acetic acid–12 N hydrochloric acid (10 : 30 : 3 v/v). The acid-treated pigment was eluted as already described. Mineral acid was removed from the pigment-free aqueous solution by washing the solution with chloroform containing 10 per cent di-*n*-octylmethylamine.

The R_F values of the purified pigment were obtained using Whatman No. 1 paper with solvent systems described for anthocyanins[6], namely, (1) *n*-butanol–2 N hydrochloric acid (1 : 1 v/v); (2) *n*-butanol–acetic acid–water (4 : 1 : 5 v/v); (3) water–12 N hydrochloric acid (97 : 3 v/v); (4) water–acetic acid–12 N hydrochloric acid (85 : 15 : 3 v/v). In addition R_F values of the pigment were determined using the original acidified methanolic extract of fresh galls. With all 4 solvent systems the R_F values of the pigment obtained from the original extract and the purified pigment were similar, showing that there had been no decomposition during the various purification stages. The R_F values of the purified pigment obtained after heating with 2 N hydrochloric acid were determined with reference to a sample of cyanidin chloride using the solvent systems recommended for anthocyanidins[6,7]. These were (1) and (2) as here, (5) formic acid–12 N hydrochloric acid–water (5 : 2 : 3 v/v) and (6) water–acetic acid–12 N hydrochloric acid (10 : 30 : 3 v/v). Mixtures of the acid-treated pigment and cyanidin chloride were chromatographed in all the given solvent systems and did not form separate spots.

Spectral measurements of the pigment before and after acid treatment were made in the visible region as described by Harborne[5] using a Unicam *S.P.* 500. The λ_{max} for the original pigment in methanolic hydrochloric acid was 527 mμ (quoted value for cyanidin-3-monoglucoside 525 mμ[5]), for the pigment after acid-treatment 535 mμ and for cyanidin chloride 535 mμ. On the addition of 3 drops of a solution of anhydrous aluminium chloride in ethanol (5 per cent, w/v) to the cell solution a shift in the λ_{max} was obtained of 19 mμ for the acid-treated pigment and 18 mμ for cyanidin chloride. This shift shows the compound to have *o*-dihydroxyl groups. The λ_{max} was also determined using as solvent ethanolic hydrochloric acid. The λ_{max} for the unknown and for cyanidin chloride was 545 mμ.

From chromatographic and spectral characters it was concluded that the pigment obtained after acid treatment is cyanidin, this being confirmed by the distribution and oxidation tests, and the tests with the cyanidin and delphinidin reagents, sodium acetate and ferric chloride (Robinson's method[8]).

From the chromatographic data obtained it was concluded that the red pigment of the galls is either cyanidin-3-monoglucoside or cyanidin-3-monogalactoside. The free sugar obtained after acid hydrolysis of the antho-

Table 1. R_F VALUES OF THE PIGMENT FROM *S. fragilis* LEAF GALLS BEFORE AND AFTER ACID TREATMENT

	(1) BuOH/ 2 N HCl (1 : 1)	(2) BuOH/ AcOH/H_2O (4 : 1 : 5)	(3) H_2O/ 12 N HCl (97 : 3)	(4) H_2O/AcOH/ 12 N HCl (85 : 15 : 3)	(5) Formic acid/ 12 N HCl/H_2O (5 : 2 : 3)	(6) H_2O/AcOH/ 12 N HCl (10 : 30 : 3)
Unknown pigment*	0·24	0·37	0·06	0·25	—	—
Unknown pigment after acid treatment	0·70	0·68	—	—	0·21	0·50
Cyanidin chloride	0·69	0·68	—	—	0·22	0·50

* R_F values quoted (ref. 6) for cyanidin-3-monoglucoside are: (1) 0·25, (2) 0·38, (3) 0·07, (4) 0·26; and for cyanidin-3-monogalactoside: (1) 0·24, (2) 0·37, (3) 0·07, and (4) 0·26.

cyanin was investigated by chromatography on Whatman No. 1 paper using ethyl acetate–water–pyridine (2 : 2 : 1 v/v), (upper phase), a development time of 26 h, and aniline phthalate (Merck) as the locating reagent. The unknown sugar had the same R_F as glucose, and separated from galactose but not from glucose. The same results were obtained when the sugar was chromatographed on thin layers of kieselgel *G* (Merck) using the solvent system of Pastuska[9], methyl ethyl ketone–methanol–acetic acid (3 : 1 : 1 v/v). The spray reagent used was anisaldehyde–conc. sulphuric acid–95 per cent ethanol (5 : 5 : 90 v/v), followed by heating at 100° C for 10 min. Glucose and the unknown sugar gave a blue colour with the spray reagent, whereas galactose gave a greenish grey, and it was concluded that the sugar obtained from the glycoside is glucose. The latter was confirmed by preparing the osazone which was identical with glucosazone.

The foregoing results, therefore, show that the red pigment in freshly collected galls of *S. fragilis* is cyanidin-3-monoglucoside. The pontanin isolated by Nierenstein and Swanton, having been obtained from dried galls, could have been formed during drying and was not the natural red pigment.

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Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity

THE reports on passive transfer of delayed hypersensitivity not only by intact cells but also with cellular extracts[1–6] have initiated studies regarding the nature of the substance responsible for this effect[2,4,5,9]. Using free electrophoresis according to Tiselius, it has been shown[5] that peritoneal-cell extracts from guinea-pigs with delayed hypersensitivity contain a protein fraction moving as the alpha-1 globulins. This protein was absent in extracts from the control animals. With this fraction it was possible to transfer passively the delayed hypersensitivity.

The aim of this investigation was to analyse the protein pattern of lymphoid-cell extracts from guinea-pigs sensitized to 2,4-dinitrochlorobenzene (DNCB) and from normal animals. The main interest was focused on the comparison between the lymph nodes regional to the sensitization and normal lymph nodes, but other lymph nodes from the sensitized animals were also studied.

In the first step of the investigation electrophoresis in starch gel[7] in a discontinuous buffer system[8] was used. Ten DNCB-sensitized animals and six control animals provided the material. The experimental set-up was essentially the same as that described below for the disc electrophoresis. By electrophoresis in starch gel it was not possible to discover any differences in the extracts from the different lymph nodes. This is in accordance with the negative results with human leucocyte extracts separated on cellulose acetate strips[9].

In the second step disc electrophoresis[10] was employed. Thirty-six white guinea-pigs weighing 300–450 g were used. Sixteen of these served as normal controls, twenty were sensitized epicutaneously with DNCB (20 vol. per cent in acetone, two applications with a 24-h interval). After a test dose of 9 μg DNCB in acetone per square cm all the animals showed positive reactions (erythema and sometimes oedema). The tests were performed on the eleventh day after the first application of DNCB and the animals were killed on the thirteenth day. The lymph nodes were homogenized in a cooled Potter–Elvehjem homogenizer. After freezing–thawing six times the samples were centrifuged in a Beckman Spinco 'Microfuge' for 4 min. The supernatants were mixed with crystals of sucrose to a final concentration of 5 per cent, and could then be used for electrophoresis. This was carried out according to a modification of the disc electrophoresis technique developed in our laboratory. Instead of using a large-pore gel of 2·5 per cent polyacrylamide on top of the column, an anticonvection medium consisting of 'Sephadex *G*-200' sedimented in 20 per cent sucrose was used and mixed with the sample. The electrophoretic procedure is shown schematically in Fig. 1. Electrophoresis was first carried out in gels of 7·5 per cent concentration for 30 min at 3·3 m.amp. Sections were cut out of the gels and transferred to the top of a column 6·8 mm wide containing 7·5 per cent gel and carefully homogenized with a glass rod. The end of the column was covered with a 'Visking 18/32' cellulose membrane. Thus there was a little space of about 300 μl. between the lower end of the gel and the membrane, which, after electrophoresis for 2 h at about 3 m.amp, contained the non-dialysable proteins from the gel sections originally placed on the column. This specimen could be additionally concentrated about three-fold by stirring dry 'Sephadex *G*-25' into the solution until a thick suspension was obtained. The gel grains were removed by centrifugation. The supernatant was mixed with sucrose and layered above a 6·8 × 40 mm column containing 15 per cent polyacrylamide gel. Even this column was charged with an anticonvection medium of 'Sephadex *G*-200'. Electrophoresis was carried out using a current of 3·0 m.amp for 100 min. Staining was performed in 0·1 g per cent amido black in 7 per cent acetic acid.

In the first electrophoretic run (7·5 per cent gel) fifteen bands were separated in extracts from lymph nodes of whatever sort. However, the band corresponding to albumin in extracts from the nodes regional to the sensitization appeared to be more concentrated than that of the control nodes. This band was further resolved in the run in the 15 per cent gel; in samples from the hypersensitive animals four distinct bands appeared compared

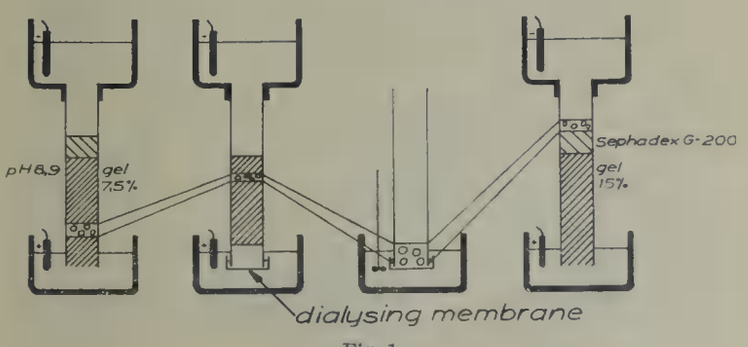

Fig. 1

Fig. 2. Pherogram of lymph node extract from a normal animal (left) and a DNCB-sensitized animal (right). Two fast-moving components are visible ahead of the albumin (strong band) in both extracts from normal and DNCB-sensitized animals; the latter show a specific fraction with an electrophoretic mobility lower than that of albumin

with three bands from the control animals (Fig. 2). It was also possible to visualize the fourth band by electrophoresis in 15 per cent gel at once without the preceding runs in 7·5 per cent gel. However, the separation was not very good in this case. The pre-albumins, also separated, have been shown to vary a little between the animals, possibly due to genetic differences.

In this investigation extracts from lymph node cells regional to the place of sensitization were used. A very weak fourth band was also found in extracts from the lymph nodes regional to the test place, while in extracts from the neck lymph nodes of the same animal this band was barely discernible. In other experiments we recently have found that the fourth band seems to be present at the highest concentration about a fortnight after sensitization. This coincides with the maximum lymphocyte infiltration in the epidermis of the test area[11]. To exclude a possible effect of the small quantity of serum left in the nodes, the sera from the animals were also separated using both the electrophoretic methods. No differences between normal and sensitized animals were seen.

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Note added in proof. During the course of publication the protein fraction described here has been further analysed and found to be an IgA (= beta-2A) globulin.

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Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulins

Recent studies have shown that cationic proteins from leucocytes produce tissue injury and responses characteristic of immunological reactions[1-4]. In an earlier publication we put forward the hypothesis that homograft rejection may be caused by such cationic proteins[5]. The specificity of the homograft reaction could only occur if the basic protein were transported to the homograft site via specific antibody (globulin) or in cells which had specificity through some chemotactic response of the cell to antigen.

This report presents a new technique in disc electrophoresis for the purpose of demonstrating the formation of a complex between basic proteins and purified globulins of serum. The method is a general one which may be applied to other complexes, but is described below from the standpoint of complexes involving plasma globulins.

The technique combines the standard system of disc electrophoresis[6] which resolves anionic proteins with a reversed system[7] that resolves cationic proteins. Resolution in the first system frees the complex (anionic) from any uncomplexed cationic component. The total protein migrating in this system is then transferred, in its gel, to the sample layer of the reversed system. Depending on the *p*H of the second or reversed system and the stability of the complex at that *p*H, resolution will show the cationic component of the complex. Any such protein appearing at this final stage must have been brought into the lower gel of the original sample tube on a 'carrier', complexed with a globular protein.

The *p*H of the sample gel as well as the 'running *p*H' of the first system must be those at which the complex is stable, whereas the same *p*H values in the second system would be those at which the complex is split into its components. These considerations led to a modification of the standard disc electrophoresis system regarding the *p*H of the various gels employed. This system normally resolves anionic substances having free electrophoretic mobilities in the range $-0{\cdot}6$ to $-7{\cdot}5$. The new system is specifically designed to give the greatest resolving power in the mobility range $-0{\cdot}75$ to $-1{\cdot}0$ which includes the slow α-, slow β-, and γ-globulins. The *p*H of the sample gel has been raised from a slightly acid *p*H of 6·9 to a *p*H of 7·3, which closely approximates to that of whole blood. The *p*H at which electrophoresis actually occurs is reduced from 9·5 to 8·6, thus maintaining a mildly alkaline medium for the sample at all times.

The changes made are only in the amounts of *tris* employed in the upper gel, lower gel and buffer. The new values for 100 ml. of solutions *A* (*p*H 7·2), and *B* (*p*H 7·3) are 10·65 g and 6·0 g, respectively, and the buffer quantity for 1 l. is changed from 6·0 g to 10·0 g (*p*H 8·5). All other solutions as well as techniques for gel formation and processing are as adopted at present. Because of the selectivity of this system for molecules of high molecular weight, it is recommended that gel concentration of 15 per cent and higher be used to effect the greatest possible resolutions.

In practice, the technique is carried out as follows. The two components of the complex to be tested are allowed to conjugate and they are then polymerized into the sample gel of the first system. No indicator dye is used because of the possible combination of the dye with one of the constituents of the complex. The ionic boundary is identified during the run by noting the difference in the refractive index between the upper and lower solutions. When the eye is placed on the level of this front it can be plainly seen. The ionic front is allowed to migrate into the lower gel only a short distance. The lower gel containing the protein is removed from the glass tube and the upper portion, containing the sample, is cut off with a sharp knife. This gel is then transferred, with the same orientation (not inverted), to the sample layer of a tube made up in the reverse system. It is covered with

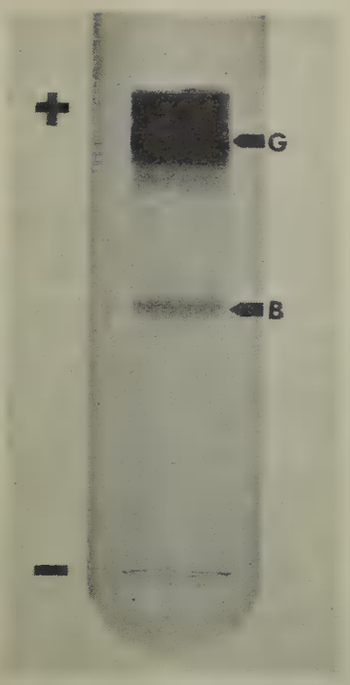

Fig. 1. Acrylamide-gel disc electrophoretic separation of basic (cationic) protein (*B*) from the carrier, α-globulin (*G*). The orientation of migration is shown on the left side of the gel

upper gel solution, all trapped air bubbles removed and then polymerized into the tube. When the ionic boundary has entered the lower gel, the hard sample gel may be removed using a bent syringe needle or hook. The boundary is allowed to migrate a previously measured distance, the gel removed from the tube, stained and de-stained in the usual manner.

Fig. 1 shows the separation of basic protein (*B*) carried as a complex with α-globulin fraction (*G*) by the technique described above. The basic protein was isolated from polymorphonuclear leucocytes of rabbits using the method of Cohn and Hirsch[8]. The α-globulin was obtained commercially from International Chemical and Nuclear Corporation, City of Industry, California. The purity of this material was checked by disc electrophoresis prior to use.

Similar experiments were conducted in which protamine was substituted for the basic protein of the leucocyte. No protamine complexed with the α-globulin in the technique previously described.

The results of these experiments clearly demonstrate the transporting of basic (cationic) proteins from polymorphonuclear leucocytes on globulin molecules. These observations become extremely provocative in the light of other work which has demonstrated that basic protein from a similar source has bactericidal activity[2] and also provokes inflammatory-like reactions[1]. Such complexes would facilitate an accumulation of toxic substance at the site of antigen-antibody interaction.

A question immediately arises whether the complex of the cationic protein and globulin remains after the globulin reacts with antigen of foreign tissue. Experiments are under way to determine the toxicity of the complex versus the free basic protein.

A possible mechanism by which one might get dissociation of such complexes would be through discharge of electrostatic attraction at the time of globulin-antigen combination. An alternative method for the dissociation could be proposed if one assumes that the low molecular weight basic protein exists as a helix. Helical configurations have been shown in various peptide chains[9–11]. Cohn and Hirsch have shown that the basic proteins exist as granules in polymorphonuclear cells and are released whenever the cell *p*H drops to approximately five[12]. Granule release at such a *p*H might occur if the protein configuration changed from a helix to a random coil.

The formation of a random coil might present such spatial differences that the globulin-cationic protein complex would dissociate. Since no complex with protamine could be demonstrated, structural differences could be assumed to have importance and not simply the basicity of the molecule.

We have already shown that removal of basic proteins from the peritoneal cavity with a Millipore 'trap' increases homograft survival[5].

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Note added in proof. Since submitting this article for publication, additional experimental details covering the electrophoresis of ionic complexes have appeared[13].

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PHYSIOLOGY

Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen

In the majority of investigations concerning the mechanism of retention of circulating noradrenaline by tissues, the amine has been administered by intravenous injection. Noradrenaline itself, as well as a wide variety of physiological and pathological conditions, are known to exert profound effects on the flow of blood through tissues. It is important, therefore, to establish the effect of changes in flow rate on the uptake of circulating noradrenaline or that liberated by sympathetic nerve stimulation. The evidence in the literature on this point is conflicting. It has been shown that the greater the flow of blood through the organ, the greater is the amount of noradrenaline in the venous effluent following stimulation of the postganglionic sympathetic nerves to spleen[1–3], intestine[4] and skeletal muscle[5]. On the other hand, spontaneous changes in flow rate have been reported to be without effect on the retention of noradrenaline by the perfused heart[6] and the perfused spleen[7]. Consequently, we have examined the effect of different, controlled flow rates on the uptake of tritiated noradrenaline by the perfused spleen.

Spleens of cats were perfused by means of a technique similar to that recently described by Fischer *et al.*[8]. However, the procedure differed in the following respects: the intestines were left intact while both the left gastric and hepatic arteries were ligated and the coeliac artery was cannulated. The spleen was perfused, at a constant flow rate, with oxygenated Krebs solution at 37° C, by means of a 'Sigmamotor' pump (model *TM*11). Perfusion pressure distal to the pump was monitored continuously using a Statham *P*-23*A* pressure transducer, the output of which was displayed on a Grass model 5 polygraph. The spleen was kept warm with cotton pads soaked in mineral oil at 37° C. The splenic nerves were cut immediately prior to the infusion of tritiated noradrenaline. Chromatographically pure tritiated *dl*-noradrenaline as a solution of the hydrochloride (New England Nuclear Corp.), with a specific activity of 7·6 c./mmole, was infused into the tubing leading to the perfusion pump using a Harvard infusion pump (model 600–910). The rate of infusion of

the amine was such as to ensure that the concentration of tritiated noradrenaline was 4·8 mg/ml. regardless of the flow rate. At each flow rate used, a total of 50 ng tritiated noradrenaline base was infused. Each spleen was perfused at least three times with the labelled amine, the first and last perfusion always being at the same flow rate. The venous effluent was collected for 2 min before, during and for 15 min after the infusion of tritiated noradrenaline, each sample being collected for 1 min. To each sample, one-tenth of the volume of 4 M perchloric acid was added. Fifteen minutes later the samples were centrifuged in a clinical centrifuge. In each case, the supernatant fluid was decanted, its volume measured, and a 0·1-ml. aliquot mixed with 10 ml. Bray's phosphor. The total radioactivity of the aliquot (^{3}H-total) was then determined in a Packard 'Tri-carb' liquid scintillation spectrometer having an efficiency for tritium of 25 per cent. Quenching was determined by the internal standard method using tritiated water. Results are corrected for quenching, which ranged from 20 to 25 per cent. Retention of tritiated noradrenaline was taken as the difference between the total amount of tritiated noradrenaline infused (assessed by direct measurement of tritium) and the total amount of tritium recovered in the venous effluent during and for 15 min after the infusion. At least 75 per cent of the tritium recovered was found in the venous effluent within 6 min of terminating the infusion.

The results of these experiments are shown in Table 1. The amounts of tritiated noradrenaline retained in the spleen during the first and last perfusions at the same flow rate were very similar. With increasing flow rates significantly decreased amounts of total-^{3}H were retained by the spleen.

At the concentration used in this investigation the retention of noradrenaline would be dependent mainly on a saturable membrane transport mechanism[9–12]. When the noradrenaline concentration is constant, such a process would be expected to operate at a constant rate[9–12]. It follows, therefore, that when the noradrenaline was infused, its passage across the membrane should proceed at the same rate regardless of flow through the organ. In our investigation, the only experimental variable was the flow rate, alterations of which necessitated increasing or decreasing the total time during which the noradrenaline was added to the perfusion fluid. The durations of noradrenaline infusion at the different flow rates are given in Table 1. Table 1 shows that there was an inverse relationship between total retention of tritiated noradrenaline and flow rate. This finding shows that retention of noradrenaline by tissues is a function of the duration of the time during which the amine is in contact with the uptake sites. After the completion of our investigations the results of a single experiment with similar results was reported[13].

If the transport and/or diffusion mechanism operates at a constant rate, then the retention of amine per unit time should be the same regardless of blood flow provided that the concentration is fixed. When the data of Table 1 were used to calculate the fraction of the total noradrenaline retention that took place per minute (that is, the rate of retention), the relationship shown in Table 2 resulted. This reveals an apparent increase in the rate of retention with increasing flow rate. If retention, in fact, takes place at a constant rate, it may be that the higher flow rates caused the apparent increase in the rate of retention (Table 2) by virtue of their perfusing more of the splenic tissue, thus bringing the tritiated noradrenaline into contact with more sites at which its retention can take place. Perfusion pressure was only slightly elevated by increasing the flow rate from 3·5 to 14 ml./min.

Table 1. Percentage of Injected Tritiated Noradrenaline retained in the Spleen at Different Flow Rates

Flow rate (ml./min)	Duration of tritiated noradrenaline infusion (sec)	Tritiated noradrenaline retained (mean percentage ± S.E.)
3·5	180	41·2 ± 1·2 (*a*) (n = 12)
7·0	90	28·1 ± 1·6 (*b*) (n = 6)
14·0	45	21·5 ± 2·3 (*c*) (n = 4)

Weighted ± test[15]: (*a*) versus (*b*), $P < 0.001$; (*a*) versus (*c*), $P < 0.01$.

Table 2. Percentage of Tritiated Noradrenaline retained in the Spleen per Min at Different Flow Rates

Flow rate (ml./min)	Mean tritiated noradrenaline retained (percentage/min)
3·5	13·7
7·0	18·7
14·0	28·7

This investigation directs attention to the importance of fluid flow rate as a determinant of noradrenaline retention by tissues. Altered flow rates change the time available for retention of noradrenaline by binding sites, as well as the extent of functional tissue perfusion. A practical implication of this work is that a single, rapid injection of noradrenaline, by altering the blood (or perfusion fluid) flow through a tissue, may thus influence the magnitude of retention. Slow infusion of the amine would circumvent this hazard. It has also been shown that the fraction of the cardiac output delivered to an organ is another determinant of noradrenaline retention[14].

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Role of Portal Blood Supply in Liver Regeneration

It has been known for many years that in laboratory animals, such as the rat, partial hepatectomy is followed by liver regeneration. In recent years it has been generally accepted that this response is initiated by some sort of humoral mechanism[1]. This view was, however, originally based on parabiotic and serum injection experiments, the validity of which has been contested. We have therefore been led to re-examine the alternative and much older 'blood-flow' theory[3]. This postulates that after partial hepatectomy the remaining liver fragment acquires a much more generous blood supply, since it has to accommodate portal vein blood previously destined for the excised lobes. It is this over-generous blood supply which is said to act as the stimulus to hyperplasia. The strongest evidence in favour of this theory was derived from experiments on domestic fowls, by taking advantage of the anastomosis between the post-caval and

portal veins through the coccygeo-mesenteric vein. It is therefore possible to increase portal blood flow by simple ligation of the postcaval vein between the kidneys and the liver. According to the 'blood-flow' theory this should itself cause hyperplasia of the liver. Furthermore, the 'blood-flow' theory would predict that, in the fowl, partial hepatectomy would not be followed by liver regeneration, since the blood which would normally be accommodated by the missing lobes could instead return to the heart via the anastomosis. It was claimed that both these theoretical predictions were fulfilled experimentally[4]. Since the number of fowls used in these original experiments was small, and since the criteria of liver growth were very crude, it seemed worthwhile to repeat them in an improved form using mitotic frequency as an index of liver growth. For this purpose we have used 26-week-old pullets of the "Sussex Legbar" strain weighing about 1,200 g. For both operations the fowls were anaesthetized lightly with intravenous 'Nembutal', and anaesthesia completed with ether. In the partial hepatectomy the entire left lobe of the liver was removed through an incision in the left side parallel to the edge of the sternum and 1 cm above it. Ligation of the postcaval vein was performed through an incision in the right flank[4].

Table 1 shows the effect of partial hepatectomy. Before the operation the mitotic frequency is very low—less than 20 per 100,000 nuclei. After the operation it shows a rather irregular increase for the first 3 or 4 days and then appears to return to a figure not very different from the pre-operative level. This suggests that, contrary to the blood flow theory, there has been a regenerative response of a sort; admittedly less dramatic than that seen in the rat, but it must be remembered that only 40 per cent of the fowl liver was removed compared with 60–70 per cent removed in most rat experiments. Table 2 shows the effect of ligation of the postcaval vein, as compared with that of a sham operation. Both procedures give mitotic frequencies rather higher than the pre-operative figures shown in Table 1; but there is no indication that the ligation has any more effect than the sham operation. This result also is in flat contradiction to the 'blood flow' theory.

Taken together, the results shown in Tables 1 and 2 seem to deprive the 'blood flow' theory of its one apparently secure experimental support. It seemed, however, desirable to carry out an analogous series of experiments in some species which, unlike the fowl, is known to show the classical regenerative response to partial hepatectomy. We have therefore compared the effects in the dog of partial hepatectomy[5] and of the establishment of a reverse Eck fistula[6]. The latter procedure, by increasing the blood flow through the liver, would be expected, according to the 'blood flow' theory, to cause liver growth similar to that which follows partial hepatectomy. The results are shown in Table 3. Partial hepatectomy, as expected, produced a very large increase in mitotic activity demonstrable 3, 6 and 9 days after the operation. By contrast, establishment of the shunt in general produced no significant increase over the pre-shunt level. There was, however, one exception. In general, when the animals were under anaesthesia before being killed, the livers were a dark bluish colour, they appeared turgid, and the normally sharp edges of their lobes had become rounded and the surface often presented a fine nutmeg appearance. At death, the liver of animal number 6, though showing the usual rounding of the sharp edges, did not have the usual homogeneous dark colour. The left central and left lateral lobes were a bright red in contrast to the bluish colour of the rest of the liver. This colour difference disappeared after death when the liver was excised. The mitotic index in the red lobes was extremely high and of the order of that seen following partial hepatectomy (Table 3). In the rest of the liver it remained at the pre-shunt level. This anomalous result can most easily be explained on the assumption that in some unknown fashion the reverse Eck fistula had disturbed the haemodynamics of the liver in such a way that blood being delivered to the left lobes was better oxygenated than that being delivered to the right. The high mitotic frequency observed in the left lobes might well be attributable to a relative increase in the oxygen saturation of its blood supply. However that may be, it seems quite clear from Table 3 that increased blood flow does not by itself bring about growth of liver tissue.

The blood flow theory of liver regeneration would therefore seem to be untenable, at least in the form in which it was originally stated.

Table 1. Frequency of Mitoses in the Livers of Fowls before and after Partial Hepatectomy

Time between hepatectomy and death (days)	Mitoses per 100,000 nuclei At operation	At death
1	6	10
2	—	167
3	5	465
	5	125
4	14	106
5	9	14
	5	38
6	8	15
7	14	10
	4	53

Table 2. Frequency of Mitoses in the Livers of Fowls after Ligation of the Postcaval Vein or Sham Operation

Time between operation and death (days)	Mitoses per 100,000 nuclei at death Sham operation	Postcaval vein ligation
2	5, 6, 10, 6	12, 20, 34, 144
3	8, 26, 16, 37	31, 30, 14, 54, 18
5	10, 11, 17, 26, 57	78, 29, 16, 42, 24
7	23, 10, 10	63, 9, 12, 7

Table 3. Effect of Reverse Eck Fistula and Partial Hepatectomy on Mitosis in the Liver

Animal No.	Operation	Mitotic figures per 100,000 nuclei Before operation	3 days later	6 days later	9 days later
1	Reverse Eck fistula	5	6	—	—
2	Reverse Eck fistula	55	13	—	—
3	Reverse Eck fistula	3	7	—	—
4	Reverse Eck fistula	18	0	—	—
5	Reverse Eck fistula	7	—	6 + 529*	—
6	Reverse Eck fistula	47	—	34	—
7	Reverse Eck fistula	3	—	10	—
8	Reverse Eck fistula	3	—	—	15
9	Reverse Eck fistula	5	—	—	5
10	Reverse Eck fistula	7	—	—	8
11	Partial hepatectomy	3	382	198	381

* Biopsy from left lobe (see text).

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Chemotopic Organization in the Bulbar Gustatory Relay of the Rat

In the relay nuclei of the mammalian auditory system, one representation of sound frequency is the location of maximum neural activity within a nucleus. This spatial representation of stimulus quality is called tonotopic organization[1]. In the gustatory system, change in the response magnitudes to chemicals as a function of change in tongue region stimulated has already been demonstrated in the nucleus of the fasciculus solitarius (NFS)[2]. The preliminary data to be presented here indicate that discrete sub-areas which are highly responsive to some chemicals are located in the anterior tongue zone of the bulbar gustatory relay of the rat. The recording and stimulating techniques have been described previously[2,3].

The active electrode was a nickel-chrome wire, 25·4μ in diameter, insulated except at the tip. The multi-unit neural activity was led through an electronic summator[4] into a recording milliammeter. The stimuli were liquids which flowed over the anterior tongue through a tongue chamber.

In the first experiments, neural responses were recorded at two depths along a single vertical electrode track in the bulbar gustatory relay. The order of response magnitudes at the more dorsal recording loci was: 0·1 M NaCl > 0·005 M HCl > 0·1 M KCl > 0·01 M NaCl > 0·01 M quinine hydrochloride (QHCl) = 0·1 M sucrose. In contrast, at the more ventral loci, the order was: 0·1 M NaCl > 0·005 M HCl > 0·1 M sucrose > 0·1 M KCl > 0·01 M NaCl > 0·01 M QHCl (summed ranks from seven animals). Note that sucrose moved from least effective at the more dorsal loci to two from the top at the more ventral loci. These different orders of stimulus effectiveness were each, for the seven animals used, statistically consistent: dorsal, $W = 0{\cdot}35$, $P < 0{\cdot}05$; ventral, $W = 0{\cdot}61$, $P < 0{\cdot}01$ (Kendall's W, ref. 5). However, the differences between the magnitudes of the more dorsal and the more ventral responses (taken relative to 0·1 M NaCl at each depth) were significant only for sucrose and for QHCl (Fig. 1) ($P < 0{\cdot}05$, Wilcoxin signed rank (WSR), ref. 5). The more ventral sucrose response was larger in all but one animal; the QHCl response was smaller in all but one. The bulbar surface co-ordinates for the electrode tracks in these animals correspond to the location of the NFS.

The two-depth responses were next studied, using a range of sucrose concentrations. Fig. 2 shows that the absolute magnitudes of the more ventral sucrose responses were larger than those of the more dorsal responses ($P = 0{\cdot}025$, WSR, ref. 5). Therefore, the previously

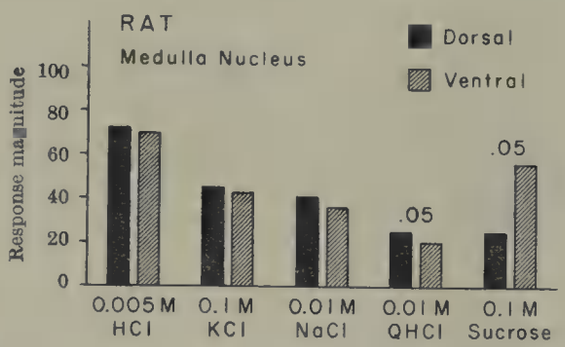

Fig. 1. Median summated relative response magnitude (response to 0·1 M NaCl at each depth, 100) at two electrode depths along a single vertical puncture in the bulbar gustatory relay. Median separation: 50μ based on seven rats. Anterior tongue stimulated by each of the five chemicals shown, and by the 0·1 M NaCl reference standard

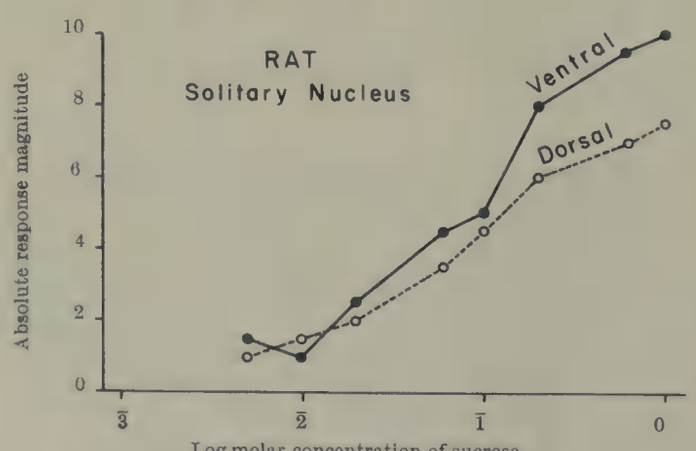

Fig. 2. Absolute response magnitudes recorded at two depths along a single electrode track to sucrose solution stimulation of the anterior tongue. Done in three animals (not those included in Fig. 1), median values shown. Separation between recording depths: 50μ (median). The brain of one rat studied histologically. The electrode track marking the responsive area ended in the nucleus of the fasciculus solitarius

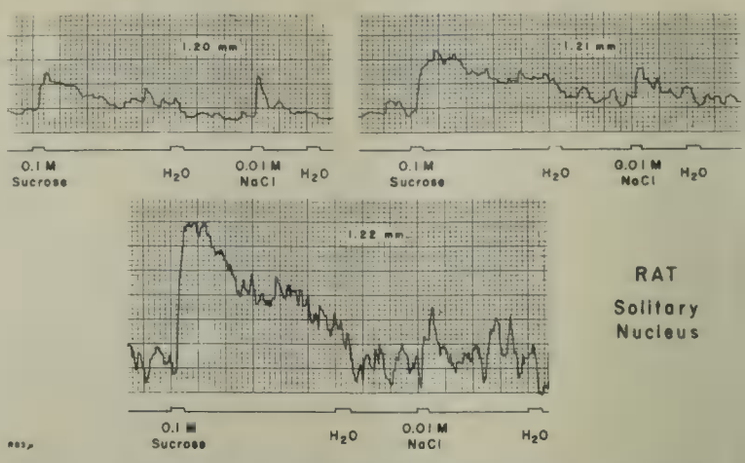

Fig. 3. Photograph of summated multi-unit responses recorded in the medulla oblongata of one rat. Records taken at 1·20 mm, 1·21 mm, and 1·22 mm ventral to the dorsal surface of the medulla. Heavy vertical time lines are 20 sec apart. Deflexion in signal marker below each record indicates approximate onset and duration of flow of each liquid. Tongue chamber empty of liquids except during stimulus flow. Records read from left to right. Critically damped, rectilinear recording milliammeter. An electrolytic lesion which was made in the responsive area was found in the nucleus of the fasciculus solitarius of the myelin stained brain. The response to sucrose is seen to double relative to NaCl; to triple, in absolute magnitude

observed increase in sucrose responsiveness with depth was not actually a decrease in responsiveness to the other stimuli. Further, the relative response magnitudes (*re*: 0·1 M NaCl at each depth) for the wide range of sucrose concentrations were significantly larger at the more ventral loci ($P < 0{\cdot}01$, WSR, ref. 5), thus demonstrating that the increase is not just a rise in the response magnitudes to all chemicals. Thus, responsiveness to sucrose increased at deeper recording sites in the bulbar gustatory relay, while responsiveness to QHCl decreased at the deeper sites.

When the recording electrode was driven through NFS in small steps, it was observed that, with an increase in electrode depth of only 20μ, the response magnitude to 0·1 M sucrose often enlarged considerably, both relative to 0·1 M NaCl and in absolute response magnitude (Fig. 3). In order to examine the highly responsive regions more closely, additional experiments were made. The electrode was driven through NFS in steps of either 20 or 25μ, and several stimuli were applied to the tongue at each step. Two patterns of response versus depth were observed (Fig. 4): a superficial, narrow area within which QHCl gave large responses, while no large responses to sucrose were recorded anywhere along the electrode track (Type *A*); and a deep, narrow area in which sucrose gave large responses, while nowhere along the track did QHCl give large responses (Type *B*). In two other animals, the response magnitudes to sucrose and QHCl were both large, separated by ≤ 21 per cent points. The highly responsive areas were narrow. In one of these animals, the maxima occurred at the same depth, half-way through the responsive area (50 per cent of total depth). In the other animal, the QHCl maximum was quite superficial; the sucrose maximum, deeper. An analysis of variance was applied to the maximum response magnitudes of the *A*- and the *B*-pattern animals, as well as to those animals whose maxima differed by ≤ 21 per cent points. The analysis indicated that the responses differed significantly for both sucrose ($P < 0{\cdot}008$) and for QHCl ($P = 0{\cdot}05$; Kruskal-Wallis[5]).

It is unlikely that the sub-areas observed are highly responsive only to sucrose or only to QHCl. However, glucose and maltose did not yield large responses in 'sucrose' sub-areas, while quinine sulphate, sucrose octa acetate, and caffeine (all bitter)[6] produced only small responses in a 'QHCl' sub-area. Thus, although indications of a chemotopic organization have been observed, the defining chemical characteristics, and many other factors, remain to be determined.

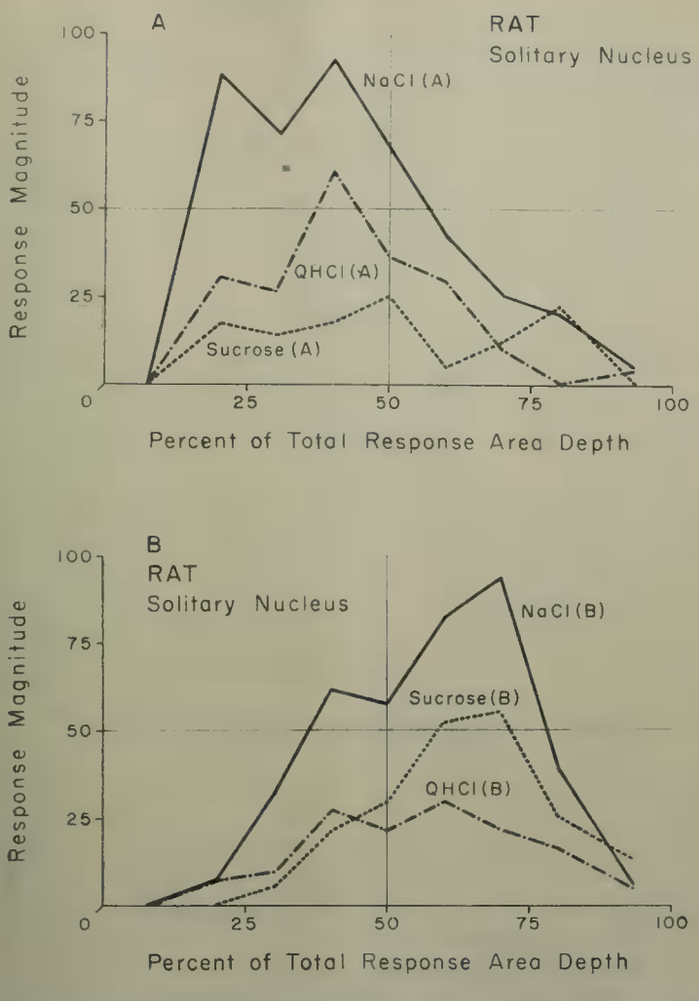

Fig. 4. Median summated response magnitudes to 0·05 M NaCl, 0·02 M quinine hydrochloride (QHCl) and 0·1 M sucrose, recorded in the nucleus of the fasciculus solitarius. Response magnitudes are expressed as percentages of the maximal response of each rat to 0·05 M NaCl. The distance from the beginning (dorsal limit) to the end (ventral limit) of the responsive area was converted into percentages (median size was 380μ). An electrolytic marker lesion was made at the recording site in each animal, and located in the anterior tongue zone of the nucleus of the fasciculus solitarius. *A*, Animals (*N*, 3) which had (1) a maximum response to QHCl $\geq$60 per cent; (2) a maximum response to sucrose $\leq$30 per cent; (3) a separation of at least 38 per cent points between QHCl and sucrose responses. *B*, Animals (*N*, 2) which had (1) a maximum sucrose response $\geq$50 per cent; (2) a maximum QHCl response $\leq$39 per cent, and (3) a sucrose-QHCl response difference of at least 25 per cent points

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Evidence for a Poisson Distribution of Miniature End-plate Potentials and some Implications

Miniature end-plate potentials (m.e.p.p.s), first discovered at the frog neuromuscular junction, have now been detected at mammalian, avian, piscine, crustacean, and insect neuromuscular junctions, at frog and chick ganglionic synapses and at frog and cat spinal cord synapses[1].

Invariably the spontaneous release of transmitter has been shown to occur at random intervals indicating no interaction between consecutive potentials. It is surprising, therefore, that the next step in such an analysis—the determination of the statistical distribution best suited to the description of the m.e.p.p. frequency—appears not to have been undertaken, particularly in view of the fact that it is generally believed that an end-plate potential represents a momentary acceleration of m.e.p.p.s, and the number of quanta in each e.p.p. evoked by nerve stimulation is distributed according to a Poisson distribution[2]. It seemed worthwhile, therefore, to determine whether the spontaneous release of quanta recorded as m.e.p.p.s also fits such a distribution. This was done in rat hemidiaphragm-phrenic nerve preparations *in vitro*. The method of dissection, the mounting of the preparation, and the bathing solution have been previously described[3]. M.e.p.p.s were recorded with microelectrodes filled with 3 M potassium chloride and frequencies were counted from photographed oscilloscope sweeps. Eight series of m.e.p.p.s were analysed (Table 1). In each, the mean frequency (m) per unit time interval (usually corresponding to one oscilloscope sweep) was calculated. The probability of finding sweeps containing $k = 0, 1, 2 \ldots n$ m.e.p.p.s was derived from the Poisson formula $p(k) = e^{-m} \cdot \frac{m^k}{k_!}$. The closeness of fit between the theoretical ($N_p(k,m)$) and observed frequencies (N_k) was determined by use of the χ^2 test with $n-2$ degrees of freedom (where n = the number of comparisons of k). The expected number of intervals for any k was usually kept greater than 5 by grouping k's. The column 'total N' shows the total number of m.e.p.p.s observed in each series. The last column in Table 1 (χ^2 level per cent) indicates the approximate percentage of ideal cases in which chance fluctuations would give a worse agreement between observation and theory.

In the first three experiments shown in Table 1, m.e.p.p.s were recorded from neuromuscular junctions in control solution. It is clear that the distribution of m.e.p.p.s in all three trials closely fitted a Poisson. At first sight, the type of distribution may appear to be of little importance, but the demonstration of a Poisson distribution has some important implications. First, it is consistent with the hypothesis that the e.p.p. is the result of synchronization of m.e.p.p.s; that is, quanta which would normally be released in periods of 50 or more sec are released in less than a millisecond under the influence of the presynaptic spike. Secondly, if it is assumed that quanta are preformed in presynaptic terminals (vesicle hypothesis), and that the probability of release is determined by the probability of collision of these quanta with the presynaptic membrane[4], then some interesting conclusions can be drawn from the fact that the spontaneous release of quanta conforms to a Poisson distribution. The release of each m.e.p.p. must be independent of past or future events, that is, the release of quanta cannot have influenced the probability of release of quanta remaining in the terminal. This implies that the population of releasable quanta remained constant, despite losses. This could occur in many ways; the lost quanta may be very rapidly replaced, or the population of releasable quanta may be so large that those lost cause little alteration of the release probability and are slowly replaced.

When the release rate of quanta was increased to 50/sec by raising the potassium concentration to 10 mM (exp. 7, Table 1), the distribution of m.e.p.p.s was still highly consistent with the Poisson hypothesis (χ^2 level 85 per cent). It has been suggested that the deviation of e.p.p. amplitude from a Poisson distribution when the quantal content of e.p.p.s is high (50–200) may be caused to some extent by an increase in the probability of release of remaining quanta[5]. It was afterwards shown that a better fit to a Poisson distribution could be obtained by

Table 1

Experiment number	Solution		Intervals with k m.e.p.p.s										Total N	χ^2 level (%)
1	Control	k	0		1		2		3		⩾4		109	75
		Observed N_k	27		38		25		9		10			
		$N_p(k; 1{\cdot}43)$	27		37·6		22·2		12·3		9·9			
2	Control	k	0		1		2		3		⩾4		144	80
		Observed N_k	64		56		18		5		1			
		$N_p(k; 0{\cdot}78)$	64·8		51		20·2		5·2		2·8			
3	Control	k	0		1		2		⩾3				117	60
		Observed N_k	54		46		14		3					
		$N_p(k; 0{\cdot}71)$	58		40·8		14·2		4					
4	Ca-free 30 min	k	0		1		2		⩾3				100	60
		Observed N_k	51		34		10		5					
		N_p $(k; 0{\cdot}7)$	49·7		34·8		12·2		3·3					
5	9 mM Ca 50 min	k	0	1	2	3	4	⩾5					79	45
		Observed N_k	12	13	23	14	9	6						
		N_p $(k; 2{\cdot}2)$	8·8	19·3	21·2	15·6	8·5	5·6						
6	16 mM Ca 50 min	k	⩽1	2	3	4	5	6	⩾7				77	60
		Observed N_k	6	10	15	20	8	9	9					
		$N_p(k; 4{\cdot}02)$	7·1	11·3	15	15	12	8	8·6					
7	10 mM K >1 h	k	⩽4	5	6	7	8	9	10	11	12	⩾13	98	85
		Observed N_k	8	5	10	12	11	18	12	7	7	8		
		$N_p(k; 8{\cdot}6)$	6·7	7·01	10·1	12·4	13·4	12·8	11	8·6	6·15	9·84		
8	HC3 45 min	k	0		1		2		⩾3				111	75
		Observed N_k	67		36		7		1					
		$N_p(k; 0{\cdot}47)$	67·2		33·6		8·4		1·4					

using a compensating factor for the non-linear summation of quanta[6]. The present results show that quantal release rates of 50/sec cause no change from a Poisson distribution of quantal release and indicate, in terms of the hypothesis of preformed quanta, that adequate refilling of the store must occur to maintain constant parameters for the Poisson.

In exp. 8, Table 1, the drug hemicholinium No. 3 (α-α-dimethylethanolamine 4,4′-biacetophenone, HC3) was used at a concentration of 4×10^{-6} M to inhibit ACh synthesis[7,8]. An attempt was then made to deplete the transmitter store in magnesium-blocked preparations by stimulating the phrenic nerve at a rate of 12/sec. Under these conditions, both e.p.p.s and m.e.p.p.s could be observed, and the quantal content of the e.p.p.s remained at approximately 10 throughout the experiment. After 45 min, when e.p.p. and m.e.p.p. amplitudes had decreased 60 per cent, which presumably corresponded to a similar reduction in the store of transmitter[8], the series of m.e.p.p.s shown in experiment 8 was analysed. It is evident that there is a remarkably good fit to a Poisson distribution in spite of the depletion of ACh. This result suggests that the population of releasable quanta remained approximately constant so that each quantum might be expected to contain less ACh, and in fact it was observed that m.e.p.p.s had become smaller in amplitude.

An alternative hypothesis[5], which fits these observations and does not depend on the postulate of preformed quanta, cannot be ignored. The probability of transmitter release may depend on the random activation of a small fraction of a large population of potential release sites in the presynaptic membrane. On the assumption that the probability of activation of potential release sites is a function of the extracellular calcium concentration[9], several series of e.p.p.s in solutions containing varying calcium concentrations were examined. In exps. 4, 5 and 6, Table 1, the calcium concentrations of the bathing solutions were 0, 9 and 16 mM respectively, but all three series were again in excellent agreement with the Poisson predictions. In several experiments with 16 mM calcium chloride, however, the χ^2 level appeared to be less than with the normal concentration of calcium chloride (2 mM). At present it is difficult to think of decisive experiments to differentiate between the two hypotheses. It has been shown that quantal size does not fall during depletion of the transmitter store during a tetanus, yet the reduced e.p.p. amplitudes still conform to a Poisson distribution[10]. In terms of the vesicle hypothesis, the reduction of quantal content implies that the store of quanta was reduced and consequently one might have expected some deviation from a Poisson distribution. The second of the two hypotheses described is more consistent with these observations.

The experiments presented demonstrate that the conformity of spontaneous transmitter release to a Poisson distribution is very significant under a variety of experimental conditions which might have been expected to affect the probability of release and thus cause deviations from this distribution. The very persistence of this conformity to a Poisson distribution must provide a powerful test of the various hypotheses of transmitter release.

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PHARMACOLOGY

Anticoccidial Activity of Nicotinamide Antagonists

THE life-cycle of poultry coccidia can be inhibited by certain *p*-aminobenzoic acid[1–3], folic acid[3–6] and thiamine[7] antagonists. This knowledge led to our testing three known antagonists of another vitamin, nicotinamide, for anticoccidial activity. These substances were 3-acetylpyridine[8], pyridine-3-sulphonamide[9] and 6-aminonicotinamide[10].

To evaluate the activity of the compounds, they were mixed in chick starter mash of known composition and fed to one-week-old cockerels, starting one day before they were inoculated orally with approximately 200,000 sporulated oocysts of *Eimeria tenella* or *E. necatrix*, or 50,000 sporulated oocysts of *E. acervulina*. In each test one infected and one uninfected control group of birds were fed unmedicated ration. The criteria to assess activity were a comparison of mortality and oocyst production[11]. The toxicity of the compounds was assessed in both infected and uninfected groups by a comparison of death rates and weight gains of the birds individually weighed at intervals.

3-Acetylpyridine at 0·1 per cent w/w in the food was inactive against both *E. tenella* and *E. acervulina*.

Pyridine-3-sulphonamide at 0·025 per cent w/w was inactive against *E. tenella* and *E. necatrix*, and showed no signs of toxicity when fed for eight days. It was active against *E. acervulina*, but less effective than sulphaquinoxaline, the reference compound (Table 1). The results of exp. 3 indicate the possibility of different strain responses. The activity was neutralized by simultaneous feeding of an equal concentration of nicotinamide (exps. 4 and 5, Table 1). In another single experiment the activity of pyridine-3-sulphonamide was not antagonized by equal concentrations of *p*-, *o*- or *m*-aminobenzoic acid.

6-Aminonicotinamide showed a different spectrum of activity to pyridine-3-sulphonamide. It was active against *E. tenella* at low concentrations comparable with those of the control drug amprolium (1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride) (Table 2), but there was little margin between the active and toxic concentrations. This nicotinamide analogue was active against *E. necatrix* and inactive against *E. acervulina* at the maximum tolerated concentration. Lower concentrations were not examined against *E. necatrix*.

Table 1. ACTIVITY OF PYRIDINE-3-SULPHONAMIDE AGAINST *E. acervulina* AND THE EFFECT OF ADDING NICOTINAMIDE

Exp. No.	Per cent drug in food P3S	NC	SQ	Strain of *E. acervulina*	Millions of oocysts passed per chick*
1	—	—	—	Ongar†	222·65
	0·0125	—	—		0·15
	0·006	—	—		10·47
	—	—	0·0125		0
2	—	—	—	Ongar†	88·26
	0·0125	—	—		0
	0·006	—	—		1·18
	—	—	0·006		0
3	—	—	—	Ongar†	113·02
	0·0125	—	—		2·14
	—	—	0·006		0
	—	—	—	Houghton‡	189·18
	0·0125	—	—		101·66
	0·006	—	—		210·75
	—	—	0·006		0·02
	—	—	—	Andover§	111·26
	0·025	—	—		0·02
	0·0125	—	—		37·60
	0·006	—	—		79·60
	0·003	—	—		85·79
	—	—	0·025		0·72
	—	—	0·0125		116·18
4	—	—	—	Ongar†	261·49
	—	—	0·006		0
	0·0125	—	—		0·76
	0·006	—	—		14·30
	0·003	—	—		78·90
	—	0·0125	—		179·68
	0·0125	0·0125	—		113·55
5	—	—	—	Ongar†	32·23
	0·05	—	—		0
	0·025	—	—		1·15
	—	0·05	—		25·03
	0·05	0·05	—		14·07
	0·025	0·025	—		22·28

P3S, pyridine-3-sulphonamide; NC, nicotinamide; SQ, sulphaquinoxaline.
* From fourth to thirteenth days post-infection.
† Isolated 1958.
‡ Isolated 1956.
§ Isolated 1960.

Table 2. 6-AMINONICOTINAMIDE ACTIVITY AGAINST *E. tenella* AND TOXICITY IN CHICKS

Exp. No.	Drug	Per cent in food	Deaths* by seventh day after infection: Acute coccidiosis	Toxicity
1	Nil (control)	—	13/15	—
	6-aminonicotinamide	0·001	0/15	13/15
		0·0005	0/15	0/15
		0·00025	11/15	0/15
	Amprolium	0·002	0/15	0/15
		0·001	3/15	0/15
		0·0005	7/15	0/15
2	Nil (control)	—	8/10	—
	6-aminonicotinamide	0·0008	0/10	0/10
		0·0004	3/10	0/10
		0·0002	5/10	0/10

* Numerator, No. of chicks dead. Denominator, No. of chicks per group.

Table 3. ANTAGONISM OF THE ACTIVITY AGAINST *E. tenella* AND THE TOXICITY IN THE CHICK OF 6-AMINONICOTINAMIDE (6-ANC) BY NICOTINAMIDE (NC)

Per cent drug in food 6-ANC	NC	Deaths* due to acute coccidiosis in infected groups after one week	Uninfected groups: Deaths* due to toxicity after 1 week	2 weeks	Av. wt. (g) gain after 1 week	2 weeks
—	—	15/15	0/28	0/28	39·3	103·3
0·002	—	0/15	15/28	21/28	5·8	20·2
0·001	—	0/10	1/20	6/20	14·0	37·1
0·0005	—	3/5	0/10	0/10	31·3	86·4
—	0·004	5/5	0/10	0/10	40·8	99·8
—	0·008	5/5	0/8	0/8	46·0	99·8
—	0·02	5/5	0/10	0/10	32·0	95·4
—	0·1	3/5	0/10	0/10	35·0	102·4
0·002	0·0005	0/5	8/10	10/10	0·6	—
0·002	0·001	0/9	4/18	15/18	3·6	17·4
0·002	0·002	0/10	7/18	13/18	14·2	46·5
0·002	0·004	0/10	2/18	9/18	15·8	67·6
0·002	0·008	4/5	0/8	0/8	33·3	81·6
0·002	0·02	5/5	0/10	0/10	32·4	93·9
0·002	0·1	5/5	0/10	0/10	32·8	92·1

Results of three experiments added together. Groups balanced for weight at the start of each test.
* As Tables 1 and 2.

The results in Table 3 show that one-half to twice the concentration of nicotinamide added to 6-aminonicotinamide has some effect in offsetting its toxicity as judged by the deaths and depression of gain in weight; but four times or more the amount of the vitamin was required to neutralize the anticoccidial activity and toxicity of this analogue.

These observations suggest that nicotinamide is a growth factor for *E. tenella*, *E. acervulina* and presumably for *E. necatrix*.

It is interesting to note that pyridine-3-sulphonamide showed activity only against *E. acervulina*, which is also generally more sensitive than *E. tenella* and *E. necatrix* to sulphanilamide derivatives. This may indicate that for *E. acervulina* transport of drugs to the site of action is enhanced by a weakly acidic group such as $—SO_2NHR$.

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PATHOLOGY

Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts

In recent years it has repeatedly been proved that neonatal or very young animals are very susceptible to carcinogenesis induced by different agents. One possible explanation is that tissues of new-born animals contain a large number of incompletely differentiated cells, particularly responsive to carcinogenic stimuli. It is relevant to this speculation that a close correlation has been noted between susceptibility of mice to develop malignant lymphomas and the presence in the thymus of a great concentration of immature cells, whether by reason of early age[1], genetic constitution[2], whole-body X-irradiation[3], or regenerative changes in implanted thymus[4]. In thymectomized, virus-inoculated[5] or X-irradiated[4] mice, implantation of new-born thymus proved much more effective than adult thymus in restoring leukaemogenesis.

Previous work showed that urethane (ethyl carbamate) in adult mice was practically devoid of leukaemogenic activity[6,7], although effective in inducing thymic changes[8]. On the other hand, administration of this chemical to new-born animals was found to provoke a significant number of malignant lymphomas[6,9]. Urethane-induced lymphomas originated in nearly all cases in the thymus, only later acquiring the character of a generalized disease.

The experiments reported here were undertaken to investigate whether, by making use of single or multiple grafts of new-born thymus as a more suitable target tissue, adult mice would be rendered susceptible to the leukaemogenic action of urethane.

C57BL male mice aged 2–3 months were grafted subcutaneously into the right flank with one or five thymuses obtained from 1- to 5-day-old syngeneic donors. Starting 5 days after implantation, they received urethane in drinking water at the 0·3 per cent level for 30 days, continuously. Adult *C57BL* mice of both sexes treated only with urethane served as controls. The total quantity of the chemical administered to animals of the different groups amounted to an average of 220 mg per mouse. A certain number of mice died within 30 days from the end of treatment, presumably owing to the toxic effect of urethane. They were not included in the investigation.

Table 1. Malignant Lymphomas induced by Urethane in *C57BL* Mice implanted with Syngeneic Single or Multiple Thymuses

Thymus graft	No. of mice in group	Mice with lymphomas No.	%
None	44	1	2
1 thymus	15	2	13
5 thymuses	22	9	41

The results, which refer to an 8-month period of observation from the end of urethane administration, are summarized in Table 1. It is evident that the highest incidence of malignant lymphomas occurred in the group grafted with 5 thymuses (41 per cent), while in animals receiving only a single thymus the incidence was markedly lower (13 per cent). However, only in the case of multiple grafts was the incidence statistically significant in comparison with the control group ($\chi^2 = 14{\cdot}15$; $0{\cdot}001 < P < 0{\cdot}01$). Neoplastic disease in all thymus-implanted animals was initiated after a mean latent period of four months as a localized growth at the graft site, and reached a maximum diameter of 40–50 mm in about 40 days (Fig. 1). Generalized leukaemia appeared later, except in three animals implanted with 5 thymuses, which at death had only local tumours. *In situ* thymus of leukaemic mice, although appearing grossly of almost normal size, showed histologically, in all cases in which the organ could be recognized with certainty, initial or limited neoplastic changes. Lymphomatous tissue at the site of implantation, as well as in the various organs involved by the generalized disease, was composed of cells of lymphoid type.

Fig. 1. *C57BL* mouse implanted with multiple syngeneic new-born thymuses and treated with urethane in drinking water. Large neoplastic mass at the graft site, 130 days after the end of urethane administration

Present data suggest that grafts of neonatal thymus make susceptible to the leukaemogenic action of urethane the otherwise resistant mice. Since in our experiments lymphomas constantly arose from the implanted thymus(es), it is possible that the positive results obtained in both the experimental groups are correlated with the availability in the graft of cells particularly susceptible to urethane-induced damage leading to neoplasia. On the other hand, the finding of the highest susceptibility in the group implanted with multiple thymuses seems to indicate that a quantitative factor is involved in the process of neoplastic transformation of the graft. Thus, it could be assumed that in a single thymus graft the number of cells sensitive to the weak leukaemogenic activity of urethane scarcely reaches the critical level necessary to initiate successfully a lymphomatous growth, under the present experimental conditions. However, the mechanism through which a greater mass of thymic tissue influences leukaemogenesis, that is, by contribution of more target cells and/or production of larger amounts of humoral substance, remains to be elucidated.

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RADIOBIOLOGY

Bone Marrow Allografts in Thymectomized X-irradiated Dogs

Allogeneic bone marrow transplantations in 1,000-r. X-irradiated dogs are therapeutically ineffective. Our earlier results[1] made it possible for us to suggest that transplanted haemopoietic cells became immunologically active against the recipient's lymphocyte antigens. All the dogs invariably indicated within 6–7 days of transplantation profound leucopenia followed by a sharp increase in white blood cell count at the cost of peculiar mononuclear hyperbasophilic cells resembling immune cell precursors or transformed lymphocytes in phytohaemagglutinin leucocyte cultures. On the next day only lymphocytes were observed in the blood; their count progressively decreased, and the animals died.

Taking into consideration previous results[2–7] which showed that adult thymectomized mice failed to restore immunogenesis after radiation and bone marrow transplantation, and that immunological competence of radiation chimeras apparently are restored by transplanted bone marrow cells which act as lymphoprecursors in thymus[8,9], we supposed that transformation of transplanted haemopoietic cells into lymphocytes in dogs also requires a thymic factor.

To prove this assumption we performed experiments on thymectomized adult dogs. Thymectomy was performed under intravenous pentobarbital anaesthesia and artificial respiration through sternal cutting. One to two months after the operation the dogs were X-irradiated with 1,000-r. (2 days running with 500-r.). The day following the last radiation the dogs were injected intravenously with $7{\cdot}4–9{\cdot}2 \times 10^9$ viable nucleated bone marrow cells in 250–300 ml. Hanks's solution. Bone marrow cells were pressed out from the vertebrae and epiphysis femur and humerus. Simultaneously the dogs were transplanted with donor's skin (the pinch graft method[10] was used). The dogs received daily intramuscular injection of 200,000 units of penicillin and 100 mg of streptomycin.

Thymectomy as such did not influence the haematological picture, in particular lymphopenia. The degree of radiation disease in these dogs was very heavy. Four dogs died 6–7 days after radiation before any re-population of the bone marrow graft appeared. At autopsy they showed large haemorrhages. Two dogs died 21 and 12 days after radiation. In one of these dogs (Fig. 1) there was sharp and quickly developing leucopenia, especially lymphopenia. However, 5–6 days after bone marrow transplantation the leucocyte count rapidly increased due to proliferation of donor cells. From the 7th day until death, donor leucocytes were identified in the blood of the dog (sex chromatin). As can be seen from Fig. 1, almost all leucocytes of this dog were polymorphonuclear. No hyperbasophilic cells appeared except during the 7th–8th days, when a small quantity of these cells was seen; scarcely any lymphocytes were present. White blood cells continued to increase—5,350 cells/mm³ on the 13th day and 13,150 cells/mm³ on the 21st day. In myelograms an insignificant quantity of hyperbasophilic cells was observed during the 7th–13th days after radiation. It should be noted that since the operation was performed on the sternum, bone marrow for investigation was taken from the iliac crest which was badly re-populated in this experiment. In spite of a good leucopoietic restoration, anaemia progressed; the haemoglobin value had fallen by the 17th day from 15·2 g per cent to 8·6 g per cent and the red blood cell count from $5{\cdot}4 \times 10^6$ to $3{\cdot}0 \times 10^6$ cells/mm³. Death of the dog was caused by renal haemorrhage. At autopsy fatty bone marrow was found in the hind legs, while in the sternum, ribs and vertebrae bone marrow was active, rich in haemopoietic red and white cell lines. Lymphoid tissues of the spleen and lymphoid nodes were atrophied. The skin graft up to the death of the dog was in good condition, and was vascularized; there were no signs of any rejection.

Similar results were indicated by the second dog. Re-population of the bone marrow graft was active. Three days later, the bone marrow was injected and there appeared, in myelogram stem cells, immature red and white cell lines which were practically absent before the bone marrow graft. Five days after transplantation the number of white blood cells increased; their amount rose from 100 cells/mm³ to 1,400 cells/mm³ in 2 days. Donor leucocytes (female in male) were identifiable up to the dog's death. The white blood cell count was fully conditioned by the increase of polynucleate cells. Hyperbasophilic cells were absent in peripheral blood; lymphocyte content was not above 2 per cent. In bone marrow few hyperbasophilic cells were observed until just before death (12th day). At autopsy hypoplasia of haemopoietic and aplasia of lymphoid tissues were seen. The haemorrhagic syndrome was insignificant, and the cause of death was not established. The skin graft was in good condition.

Thus in neither dog did we observe any significant transformation of haemopoietic cells into lymphocytes. The decisive role of the thymus in such transformations is obvious from the fact that, without any exception, in all irradiated dogs (more than 40), similarly treated but without thymectomy, clear transformation of haemopoietic

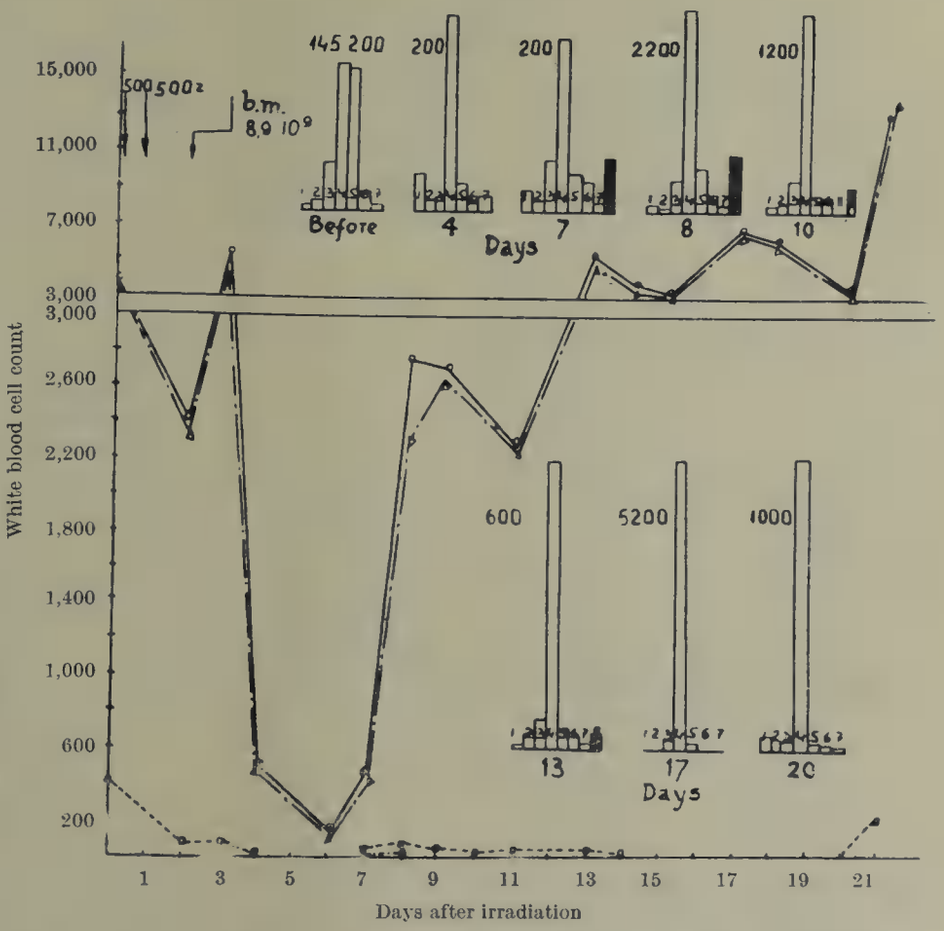

Fig. 1. White blood cell count and myelograms in a thymectomized dog irradiated with 1,000 r. On the day after radiation the dog received allogeneic bone marrow. (1) Reticular cells; (2) stem cells; (3) immature myeloid cell lines; (4) mature myeloid cell lines; (5) erythropoietic cell lines; (6) lymphocytes; (7) plasmacytes; (8) hyperbasophilic cells. Above myelograms—bone marrow cell count in 1 mm³. ○, Leucocytes; △, neutrophils; □, lymphocytes; ■ hyperbasophilic cells

cells into lymphocytes developed. These results suggest that for large animals, including man (just as for mice), the thymus is necessary for functional differentiation of polypotent stem cells into lymphoid precursor. These preliminary results indicate that even a strongly reduced thymus in adult dogs is effective. By analogy with the results already cited[2-9] one is led to believe that the thymus of adult large animals and man plays a part in the restoration of immunogenesis after radiation, 'teaching' remaining stem cells. In connexion with this assumption it seems reasonable to attempt to delay the restoration of immunogenesis (depressed by massive whole-body irradiation and cytostatics) by preliminary thymectomy prior to transplantation of tissues and organs in man.

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An Unusually Radioactive Fossil Fish from Thurso, Scotland

A plate of *Homosteus* from Clardon Haven about two miles east of Thurso was found to be radioactive to the extent of $1{\cdot}2 \times 10^4$ γ/min/g. In order to characterize the radioactive isotopes present, the energies of the emitted γ-rays were measured using a γ-scintillation spectrometer.

The spectrometer was a 3 in. × 3 in. sodium iodide phosphor used in conjunction with a photomultiplier, a high-gain amplifier and a 512-channel pulse-height analyser.

The energy range 80 keV–3,500 keV was examined and from the absorbed energies it was evident that the radioactivity was due to the decay chain of ^{238}U with some contribution from the ^{232}Th chain. The components of these chains and the appropriate gamma energies are shown in Table 1.

Table 1

Isotope	Half-life	Gamma energy (keV)
	^{238}U chain	
^{238}U	$4{\cdot}5 \times 10^9$ y	48
^{234}Th	24·1 d	29, 63, 91
^{234}Pa	1·18 min	750, 1,000
^{234}U	$2{\cdot}5 \times 10^5$ y	51
^{230}Th	$8{\cdot}0 \times 10^4$ y	67
^{226}Ra	1,630 y	188
^{222}Ru	3·815 d	—
^{218}Po	3·05 min	—
^{214}Pb	26·8 min	243, 295, 317
^{214}Bi	19·9 min	610, 1,120, 1,760 and others up to 2,430
^{214}Po	$1{\cdot}6 \times 10^{-4}$ s	Several very weak
^{210}Tl	1·3 min	
^{210}Pb	21 y	47
^{210}Bi	5·0 d	—
^{210}Po	138·4 d	800 (very weak)
^{206}Pb	Stable	
	^{232}Th chain	
^{232}Th	$1{\cdot}41 \times 10^{10}$ y	59
^{228}Ra	6·7 y	—
^{228}Ac	6·13 h	57 to 1,640 many gammas
^{228}Th	1·91 y	84
^{224}Ra	3·64 d	240
^{220}Rn	51·5 s	—
^{216}Po	0·158 s	—
^{212}Pb	10·6 h	120, 240, 300
^{212}Bi	60·5 min	40
^{212}Po	0·3 μs	280, 510,
^{208}Tl	3·1 m	580, 860, 2,620
^{208}Pb	Stable	

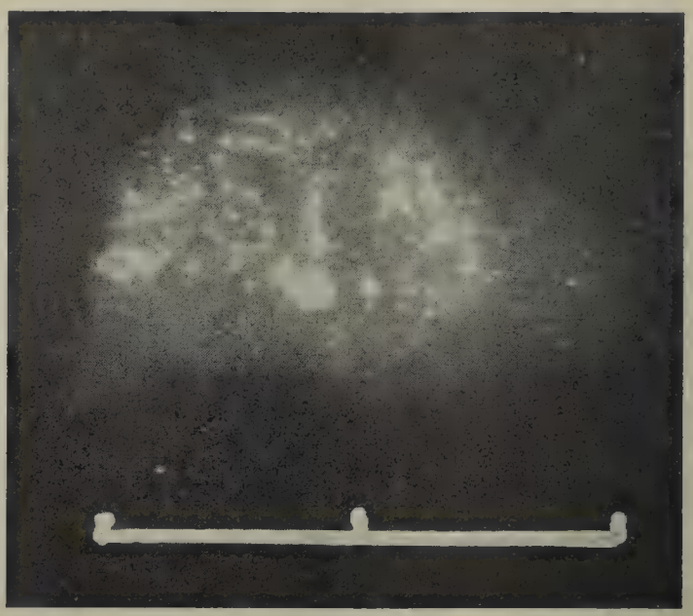

Fig. 1. Autoradiograph of a polished section of the bone of *Homosteus*. Scale, cm. The extremely bright spots are due to α-particles

To assess the distribution of activity across the specimen a section was polished and an autoradiograph was obtained using industrial X-ray film (Fig. 1). The distribution is not uniform but is concentrated into a few small areas.

A quantitative examination using the spark excited emission spectrum examined by a Hilger medium spectrograph indicated the chemical constituents as being: major, calcium; minor, strontium, phosphorus, magnesium; heavy trace, barium.

The fact that the major activity was due to the decay chain of ^{238}U differs from previous results reported by Bowie and Atkin[1], who, working on an unidentified *Homosteus* plate (*GSM* 89090), found the major activity to be due to the decay chain of ^{232}Th.

Similar examinations were made on a small specimen of *Dipterus valenciennesi* from Scrabster Brae about 1·5 miles west of Thurso and on a specimen of *Thursius pholidotus* from the shore of Thurso Bay about one mile west of Thurso. These specimens, of similar age from stratigraphical evidence, showed no unusual activity. It would therefore seem that we only find excessive radioactivity in those fossil fishes having heavy dermal armour, for example, the arthrodira.

The penetrating γ-photons from the $4n+2$ series can be readily observed using a sensitive portable γ-detector which could be a useful aid in locating specimens in the field.

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Continuous Irradiation of HeLa Cells at −196° C

The incorporation of a suitable quantity of tritiated water in the growth medium permits the continuous irradiation of cells cultured *in vitro*. A uniform source of β-radiation is thus provided which can be used in a variety of cell culture techniques. Some observations on the effects of continuous irradiation of HeLa cells grown as a monolayer at 37° C have already been published[1,2]. This communication reports some results of the continuous irradiation of HeLa cells at −196° C.

HeLa cells in logarithmic growth were suspended at 10^6 cells/ml. of Eagle's medium plus 20 per cent pooled human serum plus 10 per cent dimethyl sulphoxide and divided into 1 ml. aliquots. Suitable amounts of tritiated water were incorporated in the medium of the aliquots to be continuously irradiated. The aliquots were sealed in glass ampoules and cooled by the technique of Nagington and Greaves[3] down to −196° C in a liquid nitrogen refrigerator (by the courtesy of Dr. T. S. L. Beswick and Miss Ann Mostratos, Department of Virology, University of Manchester). In each experiment all the ampoules, control and treated, were maintained at this temperature for the same length of time, and the treated cells would be continuously irradiated at two different dose-rates to totals of 1,000 or 2,000 rads, respectively, in either 20 or 40 days.

At the end of each experiment the ampoules were removed from the liquid nitrogen and plunged directly into a water bath at 37° C. Each 1 ml. aliquot of cells was then diluted to 10 ml. in fresh growth medium (Eagle's medium plus 10 per cent pooled human serum) and the cell number was again determined to confirm that all the cells had survived at this stage of the experiment. Replicate single cell clonal cultures were then inoculated by the technique of Puck and Marcus[4] using the same inoculum size for all cultures, irradiated and control. After incubation for 10 days at 37° C the colonies were scored; comparison with the controls gave the fraction of cells surviving each régime of continuous irradiation at −196° C, determined from the average of six plates.

The surviving fractions are shown (Table 1) for each experiment and combined as an average percentage for each dosage régime. Although these average percentages are all lower than the controls, the only fractions which are significantly different are those after the 2,000 rads total dose given over 20 days at 100 rads/day. The variation between individual experiments is attributable to the lower plating efficiencies which follow the freezing technique. Nevertheless, one definite conclusion can be drawn from all these experiments: namely, that the large total doses of continuous irradiation had a very small effect on HeLa cells at −196° C.

In considering the interpretation of these results, the following facts are relevant. Continuous irradiation at 37° C leads to very little depression in the number of HeLa cells at dose-rates of 50 rads/day or less[1] since the irradiation damage is spread over many cell cycles, and growth of the surviving cells may conceal the small proportion of cells sterilized per cell cycle. On the other hand, with acute doses (for example, 100 rads/min) the radiation is delivered during a small fraction of one cell cycle, and growth during that time is negligible. The proportion of surviving cells after an acute dose of 2,000 rads is reduced to about 10^{-6} (leaving a negligible number of colonies to be counted in a constant inoculum experiment).

One might expect that a dose of 2,000 rads delivered at 100 rads/day at −196° C would produce the same effect as an acute dose delivered at 100 rads/min at 37° C. Cellular metabolism will be reduced to a minimum at −196° C and one would expect the accumulated 2,000 rads to produce as much damage over at least as small a fraction of one cell cycle as an acute dose at 37° C.

Table 1. Survival of the Colony-forming Ability of HeLa Cells after Continuous Irradiation at −196° C

20 days' irradiation	Experiment No.	% of control survival	
1,000 rads (50 rads/day)	1	105	
	2	54	75
	3	89	
	4	53	
2,000 rads (100 rads/day)	1	52	
	2	32	50
	3	68	
	4	49	
40 days' irradiation			
1,000 rads (25 rads/day)	1	66	71
	2	77	
2,000 rads (50 rads/day)	1	63	74
	2	85	

It is known, however, that acute radiation is less effective at lower temperatures. Stapleton and Edington[5] found the sensitivity of *E. coli* to be reduced at sub-freezing temperatures. Similar observations have been described by Webb *et al.*[6] for dry bacterial spores, and the phenomenon has been discussed in subsequent papers by Powers and his colleagues[7].

In mammalian cells (HeLa) Belli and Bonte[8] found an increase of radio-resistance by a factor of 1·25 by decreasing the temperature from 37° C to 5° C. They point out quite correctly that the phenomenon is unlikely to be due to any degree of anoxia. At 5° C, however, diffusion of radicals is so little affected that it is difficult to think of a suitable radiation chemical explanation of their observation. Dawson *et al.*[9] found a two-fold increase in radio-resistance of HeLa cells at −79° C when the mobility of free radicals would presumably be reduced.

At the temperature used in our experiments irradiation still produces ions, electrons, hydrogen atoms and free radicals in the solid matrix. The electrons will be captured by positive holes and/or imperfections in the solid matrix. The species capable of movement at a temperature of −196° C are presumably hydrogen atoms; the larger molecular fragments would be expected to be held in position. The hydrogen atoms may react with free radicals and may reduce some of the effects of radiation by reducing the number of radicals. Recombination of free radicals, especially fragments of macromolecules, may occur during the rise of temperature before thawing.

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BIOLOGY

Hatching Time of Turtle Eggs

Jayakar and Spurway[1] have reported possible bimodality not only in egg-laying time by a turtle (*Testudo elegans* Schoepff) but also in hatching time. Evidence from other species of turtles may be helpful in interpreting their observations.

Several species of marine turtles, of tropical or sub-tropical regions, are known to lay more than once per season[2–4], whereas terrestrial and fresh-water forms of temperate zones typically lay all the eggs of the year at one time[3]. Duration of incubation is dependent on environmental conditions, particularly temperature[2,3,5,6].

In my own experiments on turtle embryos over the past several years, I have found that hatching time for controls varies by only a few days for a given species even of different clutches, according to the temperature of incubation (Table 1). Within a given clutch of eggs there is an even smaller time-range. Hatchling size (carapace length) is positively correlated with egg size; in the very few instances in which the hatchlings were weighed the larger turtles were heavier than the smaller. There seems to be no pattern as to hatching sequence of large and small specimens.

Turtle hatchlings differ notably in their behaviour (cf. Carr[3] for adults of *Terrapene carolina carolina* (Linné)). Some are markedly aggressive, whereas others appear

Table 1. Temperature of Incubation and Hatching Time of Turtles

Species	Temperature*	No. of hatchlings†	Hatching day Range	Mean
Chelydra serpentina serpentina	Room (1951)	9 (2)	65–69	66·9
Chelydra serpentina serpentina	Room (1952)	23 (8)	62–67	64·7
Chelydra serpentina serpentina	Room (1959)	3 (3)	66–70	67·7
Chrysemys picta marginata	Room (1959)	6 (6)	64–68	66·5
Caretta caretta caretta	30° C	2 (1)	54–55	54·5
Chelydra serpentina serpentina	28° C	9 (3)	55–62	58·0

* Incubation of eggs in any one group was begun at one time, in June or July. Room temperature varied considerably, but averaged close to 28° C. The dishes of eggs at constant temperature were kept in a water bath.
† The number in parentheses after number of hatchlings indicates the number of different clutches of eggs represented in each group.

timid and retiring. In my investigations the eggs are incubated in glass dishes between two layers of moistened absorbent cotton[7]. At the onset of hatching observations are recorded at least every 12 h. Some newly hatched specimens of *Chelydra serpentina serpentina* (Linné) have made their way out of the dishes, off the table, out of the laboratory, and one even down a flight of steps, all within a 12-h period. Others, on the contrary, have hidden themselves under the cotton or within the egg shell and have made no observed attempt to explore even after several days.

Miss Karen Diamond of Rockville, Maryland, told me of watching a Carolina box turtle, *Terrapene carolina carolina* (Linné), make a nest and lay her eggs under a rose bush in nearby Kensington on May 10, 1963. As the summer wore on, the spot was examined frequently for signs of emerging hatchlings. Finally, near the end of September, about 140 days later, the nest, between 4 and 6 in. deep, was dug up and revealed egg shell fragments and three live hatchlings with no sign of the yolk sac on the plastron. All three were 'timid' turtles[8].

Domantay[4] reports for *Chelonia mydas*, and Caldwell[5] for *Caretta caretta caretta* (Linné), that the hatchlings usually emerge from the nest together, a fact which suggests that the more active ones open the way for the others.

In view of the foregoing evidence concerning other species of turtles, I suggest that the late emergers of *Testudo elegans* Schoepff as reported by Jayakar and Spurway[1] were not slow in developing but rather inactive as hatchlings, and that the correlation of low weight with late emergence from the hatching site is due to the yolk having been used up during the prolonged stay in the soil.

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Development of the Ovule in a Species of *Cissus*

In an investigation of the floral morphology and embryology of the Vitaceae the following features were observed in the development of the ovule of *Cissus trilobata* Lamk. collected from Changanacherry, Kerala State, India.

The ovule is anatropous, bitegmic and crassinucellate. Of the two integuments, the inner which projects beyond the outer is initiated first by periclinal divisions in the protoderm simultaneous with the differentiation of the

Fig. 1. Longitudinal section of the ovule (× 130). *oi*, Outer integument; *ii*, inner integument

ovular archesporium in the first hypodermal layer. It develops faster than the nucellus and not only encloses the latter but also grows very long, 2–3 times the length of the nucellus, into a tubular structure which is thrown into folds and coils (Fig. 1). Although it is known that in other members of the Vitaceae the micropyle is formed by the inner integument[1–4], coiling and folding of the integument have not so far been mentioned. Moreover, such a long, coiled, and folded micropylar canal is not reported in any of the flowering plants.

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Marsupial Spermatozoa Pairing in the Epididymis of American Forms

In 1962 Biggers and Creed[1] pointed out that conjugation (henceforth called pairing) of spermatozoa is a normal occurrence in the epididymides of the North American opossum (*Didelphis marsupialis virginiana* Kerr). Evidence has now been obtained which shows that this phenomenon is found in the majority of, if not all, marsupials in the American continents, and that, if it occurs in Australasian marsupials, it is likely to be exceptional. Also, three morphologically distinct types of spermatozoa have been found to exist in the extant American group, which consists of 69 species[2] grouped into 15 genera and two families. Our observations are summarized in this report.

The species of marsupial we have examined are shown in Tables 1 and 2. In most cases, fixed specimens of the testes and epididymides were examined histologically. However, the living epididymal spermatozoa of *Philander opossum* (four-eyed opossum), *Marmosa mexicana* (murine opossum), and *Caluromys derbianus* (woolly opossum) have also been examined.

Pairing of spermatozoa has been observed in the epididymides of all the species of American marsupials shown in Table 1. In no instance was pairing observed in sections of the testes. The only genera we have not been able to examine are *Gironia*, *Dromiciops*, *Caluromysiops*, *Lutreolina* and *Notodelphis*. A sufficient number of data are available on the two sub-species of *Didelphis* and *Philander* opossum to indicate the incidence of pairing within species. Out of 92 specimens of *D. m. tabascensis*, 88 (95·7 per cent) were paired, and out of 56 specimens of *P. opossum*, 54 (96·5 per cent) were paired. This incidence is very similar to that found for *D. m. virginiana*[1]. Thus the pairing of

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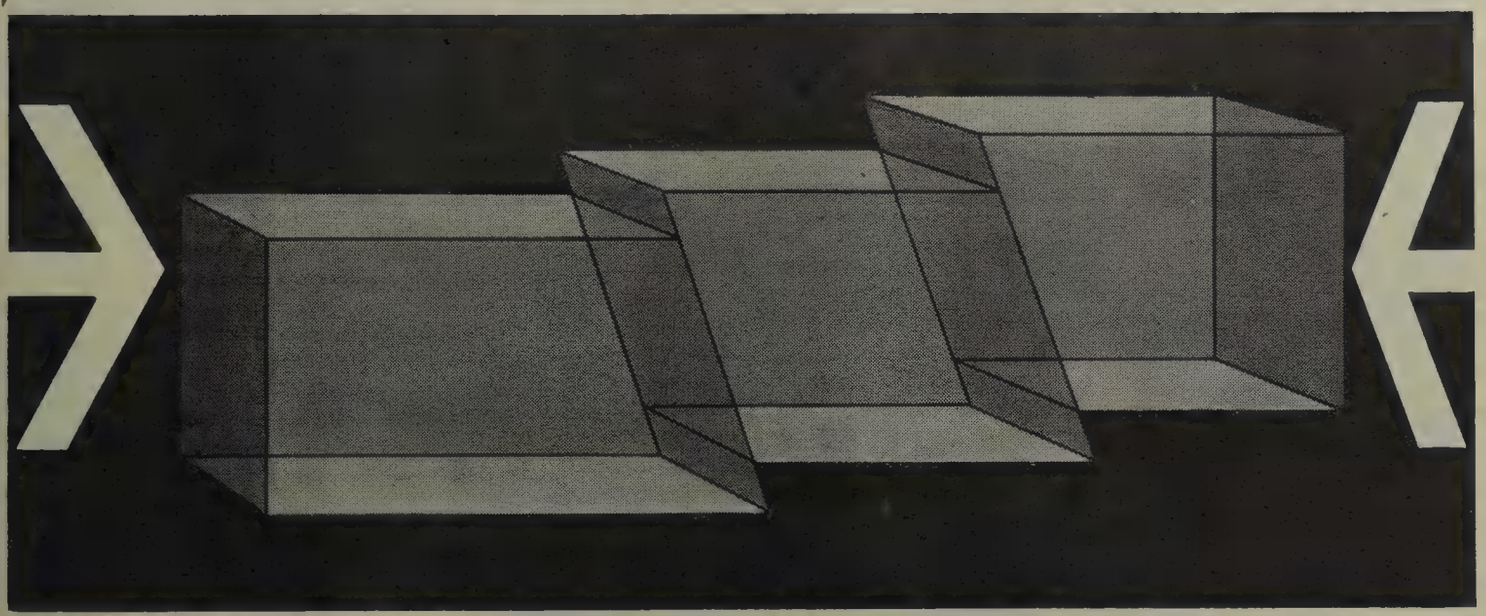

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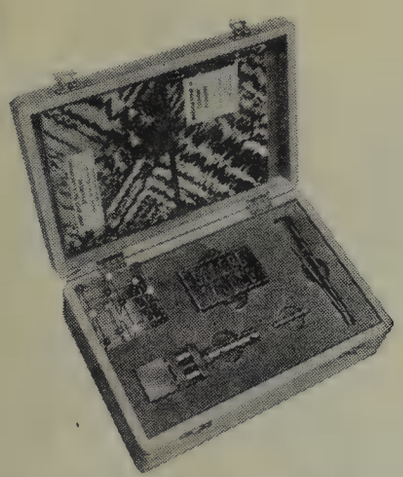

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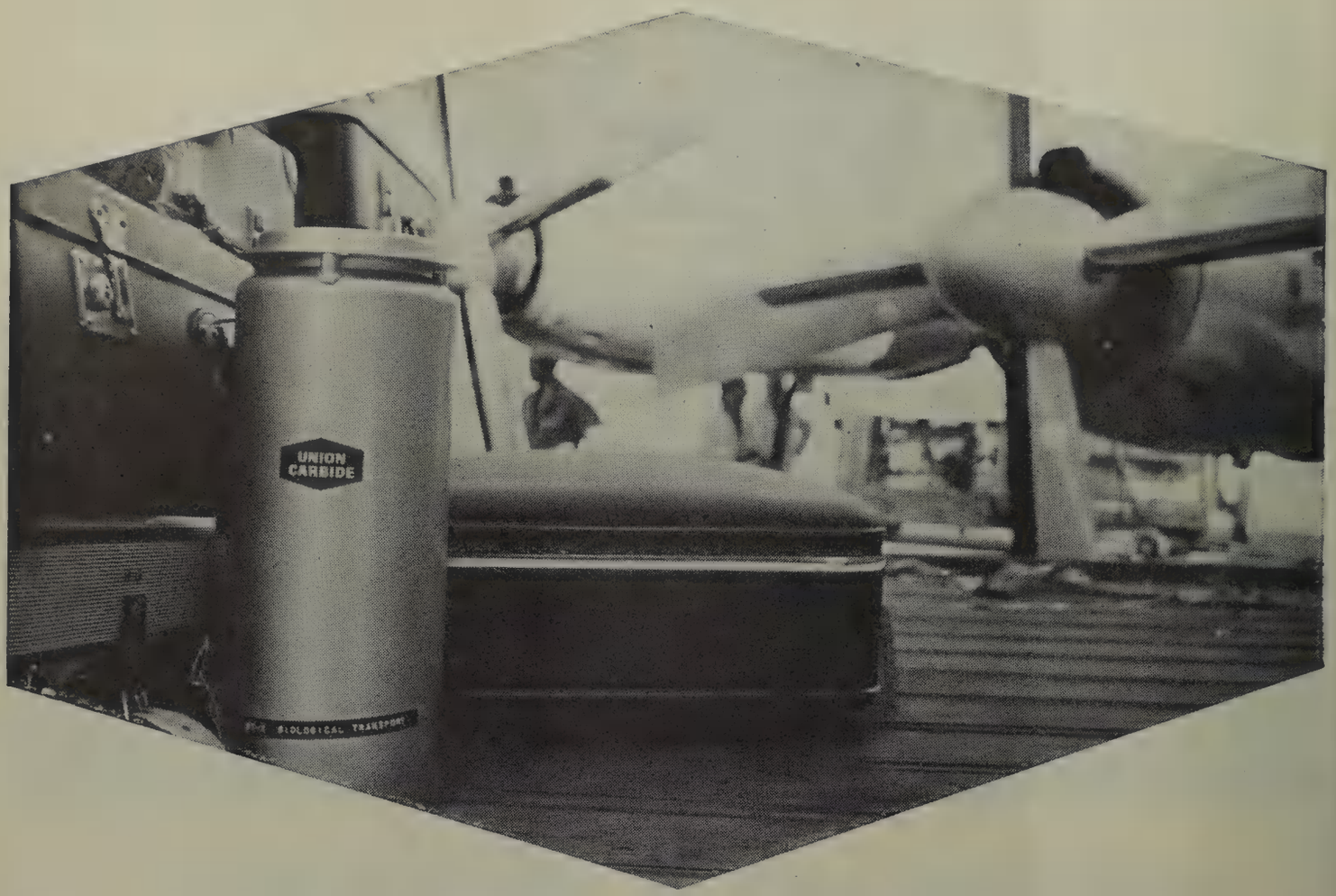

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Table 1. American Marsupials in which Epididymal Spermatozoa have been examined for Pairing. Although Several More Specimens were examined, only those unequivocally identified are included

Family	Species	Vernacular name	No. of specimens	Source
Didelphidae	*Didelphis marsupialis tabascensis*	Common opossum	88	(*a*)
	Philander opossum	Four-eyed opossum	54	(*a*)
	Chironectes panamensis	Water opossum	1	(*c*)
	Monodelphis brevicaudatus	Short-tailed opossum	1	(*b*)
	Metachirus nudicaudatus	Brown opossum	1	(*b*)
	Marmosa mexicana	Murine opossum	1	(*a*)
	Marmosa desmararae	Murine opossum	2	(*b*)
	Caluromys derbianus	Woolly opossum	3	(*a*, *d*)
Caenolestidae	*Caenolestes*	Rat opossum	12	(*b*)

See footnotes for Table 2.

Table 2. Australasian Marsupials in which Testicular and Epididymal Spermatozoa have been examined for Pairing. Although Many More Specimens were examined, only those unequivocally identified are included

Family	Species	Vernacular name	No. of specimens	Source
Dasyuridae	*Sminthopsis crassicaudata*	Fat-tailed sminthopsis	2	(*b*)
	Sminthopsis rufigenis	—	1	(*b*)
	Neophascogale lorentzi	—	1	(*b*)
	Antechinus flavipes	Yellow-footed marsupial mouse	1	(*b*)
Phalangeridae	*Acrobates pygmaeus*	Pigmy glider	2	(*b*)
	Eudromicia caudata	Pigmy possum	1	(*b*)
	Trichosurus vulpecula	Brush-tail possum	1	(*e*)
Phascolomidae	*Phascolomis mitchelli*	Common wombat	1	(*e*)
Peramelidae	*Perameles nasuta*	Long-nosed bandicoot	1	(*f*)
	Peroryctes longicauda	Bandicoot	3	(*b*, *g*)
Macropodidae	*Protemnodon bicolor*	Swamp wallaby	1	(*e*)
	Megaleia rufa	Red kangaroo	2	(*e*)
	Macropus robustus	Wallaroo	3	(*e*)
	Macropus canguru	Great grey kangaroo	1	(*e*)

(*a*) Dr. L. G. Clark, Leptospirosis Unit, University of Pennsylvania, Nicaragua.
(*b*) American Museum of Natural History, New York. Courtesy of Dr. H. van Deusen.
(*c*) Dr. W. A. Wimsatt, Department of Zoology, Cornell University.
(*d*) Dr. J. A. Davis, curator of mammals, New York Zoological Society.
(*e*) Dr. G. Sharman, Division of Wildlife Research, Commonwealth Scientific and Industrial Research Organization, Australia.
(*f*) Prof. A. S. Parkes, Department of Physiology, University of Cambridge.
(*g*) Archbold Collection, American Museum of Natural History, New York. Courtesy of Dr. H. van Deusen, curator.

spermatozoa in the epididymides is a widespread, if not universal, phenomenon in the American stock of marsupials. It is impossible at present to determine the extent of pairing in the epididymis and the degree of variation between individuals. This will only be possible when methods of collecting ejaculates from these species have been devised.

The pairing of spermatozoa in the epididymides has not been observed in any of the species of Australasian marsupial shown in Table 2, and in no case was pairing observed in sections of the testes. Thus it seems unlikely that pairing of spermatozoa occurs in the epididymides of the Australasian stock of marsupials.

During the course of this work three distinct morphological types of spermatozoa have been found in the American marsupials. These will be designated the *Didelphis*, *Caluromys* and *Caenolestes* types respectively, and pairs of them are illustrated in Figs. 1, 2, 3. *Didelphis* is the well-known type, and this has been described elsewhere[1,3]. *Monodelphis*, *Philander*, *Metachirus*, *Chironectes* and *Marmosa* all have this morphological form of spermatozoa. The spermatozoa of *Caluromys* possess saucer-shaped heads with the mid-piece inserted into the convex side, and the acrosome lying in the concave side. Pairing occurs by apposition of the two concave sides. The spermatozoa of *Caenolestes* have only been observed in fixed material. Unfortunately, this is a very rare species indigenous to localized areas of the Andes, and it is very unlikely that living material will be obtained. The spermatozoa are very rectilinear in shape with a niche on one side from which the mid-piece arises. Pairing occurs by apposition of the edges opposite the insertion of the mid-piece.

The phenomenon we have described as pairing was first observed in *D. marsupialis* by Selenka[4], who referred to it as 'copulating' spermatozoa. This name was used by Von Korph[5], who made the crucial observation that the spermatozoa arise as single cells in the seminiferous tubules, and that the pairing only occurs after the gametes reach the epididymides. Later, Wilson[6] objected to this label, and introduced the word 'conjugating'. However, this word has acquired a specific meaning in biology with reference to the union of gametes in unicellular organisms. For this reason, we prefer the simple descriptive word 'pairing' to describe the phenomenon. Perhaps, when experimental work has elucidated the phenomena involved in this highly oriented adhesion of cells, a more explicit word may be coined for its description.

The finding of three morphologically distinct types of spermatozoa in the few genera of American marsupials is pertinent from a taxonomic point of view. Osteological and palaeontological evidence was interpreted by Reig[7] to suggest that *Caluromys*, *Dromiciops* and *Glironia* are related members of the sub-family Microbiotheriinae formerly thought to be extinct. The finding that the spermatozoa of *Caluromys* are morphologically different from that of the sub-family Didelphinae may therefore be systematically significant. An examination of the sperma-

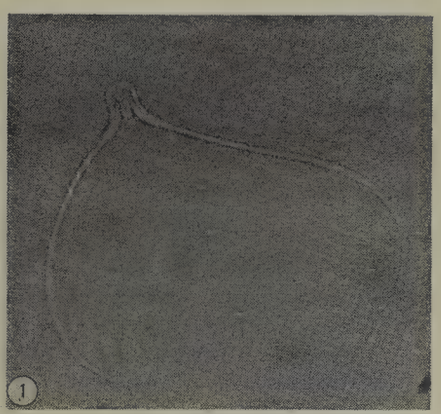

Fig. 1. Paired spermatozoa expressed from the epididymis of the four-eyed opossum (*Philander opossum*). (×805)

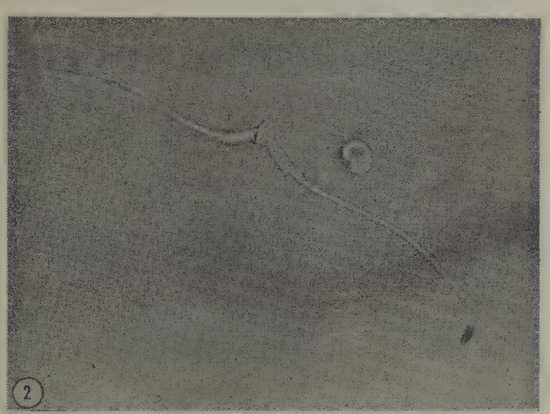

Fig. 2. Paired spermatozoa expressed from the epididymis of the woolly opossum (*Caluromys derbianus*). (×805)

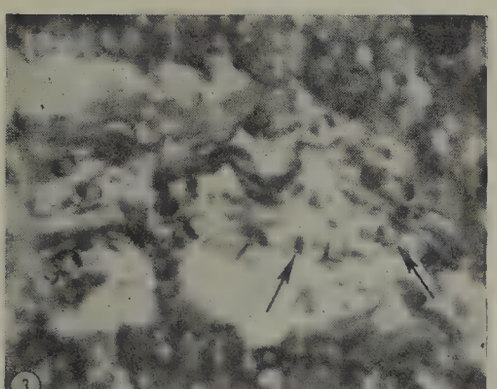

Fig. 3. Section through the epididymis of *Caenolestes*. The arrows indicate the heads of paired spermatozoa. (×805)

tozoa of *Dromiciops* and *Glironia* to see if they are morphologically similar to those of *Caluromys* would elucidate this problem, but unfortunately this has been impossible because of the rarity of these species. The observation that *Caenolestes* has spermatozoa morphologically distinct from other American marsupials is of interest because of the unusual features of this survivor of an ancient marsupial group of the Tertiary epoch[8]. These animals are unique in that they possess affinities with members of the super-family Phalangeroidea found only in Australasia, thus providing a possible connexion between the American and Australasian groups of marsupials[9]. Investigations of the morphology of marsupial spermatozoa, however, indicate no similarity between the American and Australasian forms[10], and therefore, so far as this character is concerned, there is no affinity between the two major marsupial groups.

The fact that pairing of spermatozoa has been observed in all types of American marsupial which have been examined, transcending two natural families and three morphological types, and has not been observed in Australasian marsupials, indicates that both the Caenolestidae and Didelphidae are derived from a common ancestral stock, the descendants of which evolved only in the Americas. Furthermore, pairing of spermatozoa, whatever its function, must have become established early in the evolutionary history of the group.

This work was supported in part by grant *G*-17658 from the U.S. National Science Foundation, and general research support grant, U.S. Public Health Service, *FR*-05464.

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Record of the Bivalve Gastropod *Berthelinia limax* (Kawaguti and Baba 1959) from the Indian Ocean

A living bivalve gastropod of the order Sacoglossa, found in Japan by Kawaguti and Baba[1] in 1959, was described as a new genus and species *Tamanovalva limax*. Keen and Smith[2], Baba[3] and Taylor and Sohl[4] synonymized the genus *Tamanovalva* Kawaguti and Baba 1959, with the Eocene Paris Basin fossil genus *Berthelinia* Crosse

Fig. 1. Two live specimens of *Berthelinia limax* (Kawaguti and Baba 1959) (×25)

1875 and included it under the sub-family Bertheliniinae Beets, 1949, of the family Jullidae Dall, 1898. Since then, living members of this genus have been reported from a number of other Pacific localities in Japan[5], Australia[6], California[7] and Hawaii[8], and from Jamaica[9] in the Atlantic. The purpose of this communication is to record the collection of living specimens of *Berthelinia* for the first time from the Indian Ocean region.

Recently, while examining nudibranch molluscs near the jetty of the Central Marine Fisheries Research Institute at Mandapam Camp facing the Gulf of Mannar, I came across four specimens of small bivalve gastropods, found attached to the roots of the green alga *Caulerpa racemosa* (Forsskål) J. Agardh, which on close examination resembled *Berthelinia limax* in all essential features (Fig. 1). The measurements of the four specimens are given in Table 1.

Table 1

Specimen No.	Length (mm)	Height (mm)
1	2·76	1·94
2	2·53	1·76
3	2·41	1·76
4	2·00	1·35

The habitat of *Berthelinia* appears to be restricted to various species of *Caulerpa* on which it feeds. Its known distribution along with the algal habitat is shown in Table 2.

The present specimens resemble the Pacific species, and the differences noted are very minor and not sufficient to warrant any specific separation. They are, however, distinct from the West Atlantic species, *Berthelinia caribbea* Edmunds[10], where the shell valves are green or grey-green, often tinged brownish, with more or less distinct yellow rays.

The animals are leaf green in colour with auriculated rhinophores ornamented with opaque white spots. The head, neck and sole are light green in colour, while the

Table 2

Spec. No.	Species	Algal habitat	Reference	Locality
1	*Berthelinia limax*	*Caulerpa okamurai*	Kawaguti and Baba (ref. 1)	Bisan Seto, Inland Sea, Japan
2	*Berthelinia typica*	*Caulerpa scalpelliformis*	Burn (ref. 6)	Torquay, Victoria, Australia
3	*Berthelinia australis*	*Caulerpa scalpelliformis* *Caulerpa brownii* *Caulerpa simpliciuscula*	Burn (ref. 6)	Torquay, Victoria, Australia
4	*Berthelinia chloris*	*Caulerpa racemosa* *Caulerpa sertularioides*	Keen and Smith (ref. 2)	Puerto Ballandra Bay, Baja, California
5	*Berthelinia* sp ?	*Caulerpa racemosa*	Kay (ref. 8)	Koloa, Kauai, Hawaii
6	*Berthelinia caribbea*	*Caulerpa verticillata*	Edmunds (ref. 10)	Port Royal, Jamaica
7	*Berthelinia limax*	*Caulerpa racemosa*	Present work	Mandapam Camp (Gulf of Mannar, India)

mantle and the liver are deep green. A few irregularly placed dark green spots are present on the liver. The valves of the shell are equal in size, light green in colour, and extremely thin and fragile, with a smooth surface which is marked by fine growth lines. The helicoid protoconch is translucent and of one and half whorls, attached to the posterior extremity of the left valve as in *Berthelinia limax*. The protoconch is directed backwards extending horizontally over the right valve. The position of the yellowish white circular attachment of the adductor muscle is as in *B. limax*.

One of the specimens laid an egg string which was kept under observation. The early development agreed in all essential respects with the observations of Kawaguti and Baba[1] and Kawaguti and Yamasu[11,12] on *B. limax*.

With the record of the genus *Berthelinia* from the Indian coast it could be presumed that the species of the genus are distributed throughout the coastal waters of all the warmer seas. A careful search might prove that it is widely distributed in the Indo-Pacific waters.

I thank Dr. Umamaheswara Rao for identifying the alga, Dr. P. S. B. R. James for advice, and Mr. K. G. Nambiar for the photograph.

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Spore Discharge in *Lepiota konradii*

Recent investigations[1] based on laboratory experiments have shown that ballistospore discharge is much affected by the humidity and temperature of the ambient air. The observations reported here are based on field studies during which an effort was made to determine the effect of temperature and humidity on ballistospore discharge under natural conditions.

On the morning of January 15, 1965, three fruiting-bodies of *Lepiota konradii* Huijsman ex P.D. Orton[2] were found growing in the Biological Garden, University of Ife, Ibadan, Nigeria; by noon, their pilei were beginning to expand.

An electric-driven Hirst spore trap was set up near these fruiting-bodies. The trap was placed so that the orifice was at a height of 27 cm above ground-level. The horizontal distance of the orifice from the pileus of the nearest fruiting-body was 56 cm.

Sampling at the rate of 0·6 m³/h was continued until the fruiting-bodies were found to be withering, drying, and tottering as if they were about to fall. The sampling period was January 15–18, 1965.

The ballistospores being sticky themselves, clean slides were used without any adhesive. These were changed daily at 1300 h Nigerian Standard Time. After exposure, slides were mounted for examination in glycerol and scanned under low magnification. Counts were made on cross traverses 34μ wide and 4 mm apart, representing 2-h intervals. The numbers of spores counted were then converted into an estimated number per cubic metre of air.

Records of temperature and humidity were obtained by means of a Casella thermo-hygrograph situated under the roof of the spore trap.

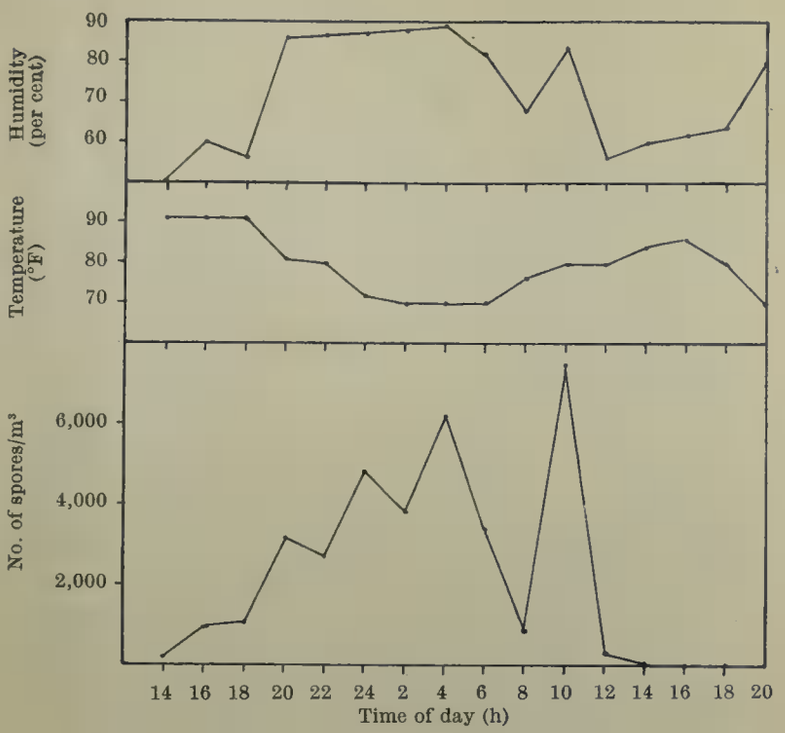

Fig. 1. Hourly mean concentration of ballistospores

The results shown in Fig. 1 clearly indicate a correlation between spore discharge and relative humidity. The increased rate of spore discharge at 1800 h and reaching a maximum (6,200 spores/m³ air) at 0400 h—a period of rapid liberation of ballistospores—corresponds quite closely with that of decreasing temperature and increasing relative humidity of the surrounding atmosphere.

Counts at 2200 h and 0200 h show, however, that the concentration of spores in the 2-h periods preceding these times had been slightly reduced. This needs further investigation, which is being planned. It was probably due to a change in the direction or the velocity of the wind, or possibly to the fact that nearly all mature spores were liberated during the early part of the period of high humidity, so that there were not many spores left for liberation during the later part. On the other hand, the maximum discharge recorded at 0400 h might have been due to a recovery in the number of mature spores.

Shortly after 0400 h the rate of spore discharge decreased gradually and reached a minimum (1,000 spores/m³ air) at 0800 h. The relative humidity then recorded was fairly low (68 per cent).

Again there was a further large increase (to 7,700/m³) in concentration of these ballistospores in the air at 1000 h. Such abundant liberation was correlated with a sudden increase in humidity caused by a slight trace of rain. Thereafter the spore liberation decreased very rapidly, so that by 1200 h the spore concentration in the atmosphere was only 300/m³ and by 1400 h liberation had apparently fallen to zero. For, although subsequent observations indicated numerous dust and smoke particles in the air, no *Lepiota* ballistospores were found impacted on the slides.

These observations also show that the spore discharge period for the fruiting-bodies investigated began at 1400 h in the afternoon and ended about 1200 h the next morning, being accordingly of about 22 h duration.

The observations reported here indicate that temperature and humidity control ballistospore discharge in the field—as in the laboratory. It would be interesting to know to what extent, if any, temperature and humidity control the development of the ballistospores.

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FORESTRY

Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic acid

The xylem formed on the upper side of arborescent angiosperms in reaction to inclination is often of the abnormal tension wood type. A complete discussion of tension wood anatomy is given in a recent review by Wardrop[1]. In brief, it is characterized by the presence of a large number of thick-walled, 'gelatinous' fibres of unusually low lignin content, and a reduction in the size and number of vessels over that of normal wood.

The reaction wood observed in leaning coniferous stems occurs on the under side. Since reaction wood in conifers can be induced to form on application of indolyl-3-acetic acid (IAA) to erect stems[2–4], the mechanism of its formation in leaning trees is generally thought to be associated with supra-optional concentrations of IAA or closely allied growth-promoting substances. However, application of IAA to the upper surface of young, bent seedlings of *Populus monilifera* prevented the formation of tension wood[4]. In this latter study, untreated inclined branches of *Populus alba* were observed to form tension wood on their upper sides in association with low levels of IAA and the presence of growth inhibitors. As a logical extension of this study, we investigated the possibility of inducing the formation of tension wood in an *erect* stem of a hardwood by applying a synthetic anti-auxin. The use of such anti-metabolites in physiological investigations of tension wood formation has also been suggested by Berlyn[5].

The anti-auxin 2,3,5-tri-iodobenzoic acid (TIBA) was selected for use. It is known to act as an IAA antagonist either through direct competition with auxin or by blocking its movement about the plant[6]. Larson[7] has employed this substance with *Pinus resinosa* to induce the formation of tracheids of reduced radial diameter, a response contrary to that evoked by application of IAA. A lateral exogenous application of TIBA in lanolin paste (1:100) was applied to a four-month-old white elm (*Ulmus americana* L.) seedling 5 mm in diameter. After first scraping the outer bark at a point 7 cm above the level of the rooting medium ('Perlite' irrigated with a complete nutrient solution), application was made in a band 5 mm wide completely around the periphery of the stem. At the conclusion of a 35-day period the stem was collected by cutting into 3-cm lengths. These pieces were aspirated in FAA solution and sectioned transversely at three different levels: (1) directly above the point of application of TIBA, (2) 2 cm below, and (3) 5 cm above this point. Sections were stained in safranin and fast green before microscopic examination.

The wood formed by the cambium as a result of application differed markedly from that produced before treatment (Fig. 1). Directly above the point of application, the mature wood formed in response to TIBA consisted of four bands of rectangular conductive cells resembling coniferous tracheids due to their orientation into tangential and radial rows (Fig. 2). These bands of conductive tissue alternated with three bands of fibres, the last of which was almost entirely gelatinous. At the widest point, the number of mature cells in a radial row through these bands totalled about 60, indicating a very rapid rate of cell production. The areas sampled above and below this location had approximately the same features, namely: a zone of response to TIBA only half the radial width of position (1); conductive tissue similar to that described above, except in shorter tangential lines; fibrous zones which were exclusively gelatinous (Fig. 3).

Equal parts of hydrogen peroxide and acetic acid were used to macerate the layer of wood formed in response to TIBA application. Examination of the pulp verified that the conducting elements seen in transverse section were vessel segments, approximately 0·13 mm long, with simple perforations and heavy spiral thickenings. The vessel elements of the normal wood were likewise simply perforated, but were often twice the length of the former, and had only weak spiral markings confined to the latewood.

The faster growth rate, reduction in size of vessel elements, and the formation of gelatinous fibres, especially in the two locations more remote from the point of application of TIBA, establish a cambial response leading to tension wood formation. The fact that the total volume of vessels was not reduced must be regarded as an anomaly. Nevertheless, these results lend support to the hypothesis that tension wood forms in response to an auxin deficiency. In naturally leaning hardwood stems and inclined branches, the auxin deficiency probably results from the influence of gravity on its distribution, leading to the conclusion of

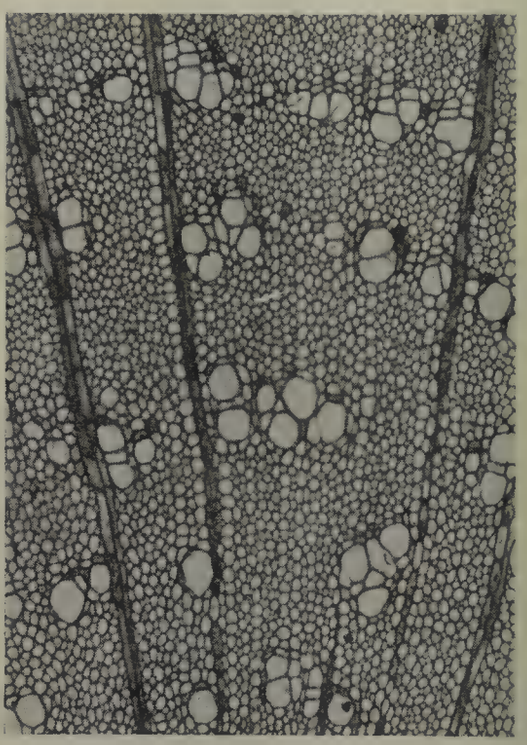

Fig. 1. Appearance of xylem formed in *Ulmus americana* stem before application of TIBA

Fig. 2. Appearance of xylem formed in erect *Ulmus americana* stem immediately above the point of application of TIBA. Gelatinous fibres were present only in the third band (top of photograph)

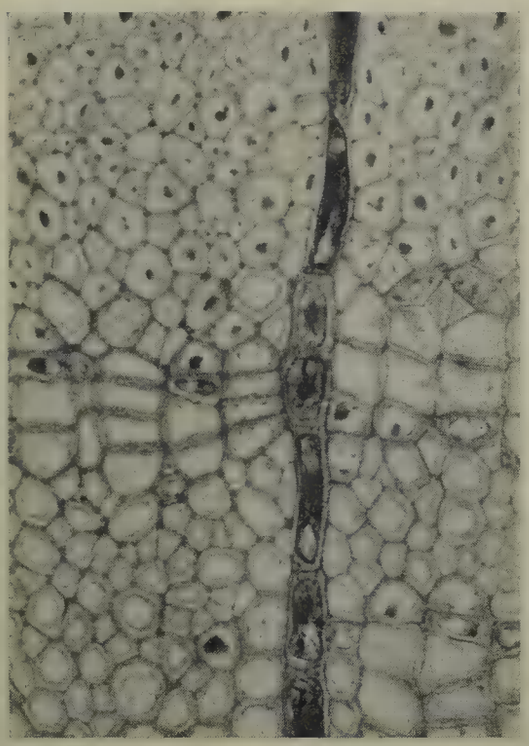

Fig. 3. Comparison of xylem fibres formed in erect *Ulmus americana* stem, 5 cm above the point of application of TIBA. The central tangential band of small vessels and axial parenchyma separates fibres formed before (lower half) and after (upper half of photograph) application of TIBA

Wardrop[1] that gravity is a major factor governing the formation and distribution of tension wood.

This study is continuing with extensive sampling of 14 angiospermous species on which TIBA has been applied to lateral and excised apical surfaces.

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Leucoanthocyanins of White Pine in relation to Weevil Attack

Leucoanthocyanins may serve as growth-promoting substances[1] as well as precursors to tannin formation[2]. Both these functions suggest that leucoanthocyanins may be involved in the complex of factors which influence pest resistance in plants. With this possibility in mind, an investigation of the leucoanthocyanins of eastern white pine (*Pinus strobus* L.) was carried out to determine the relationship between leucoanthocyanin content and susceptibility to white pine weevil (*Pissodes strobi* (Peck)).

In the only previous report dealing with leucoanthocyanins in *P. strobus*[3], both leucocyanidin and leucodelphinidin were found in needles and bark, while the wood contained only leucocyanidin. No quantitative data were given in that report.

Parent trees of the control-pollinated progenies used in this investigation were located at the Morris Arboretum of the University of Pennsylvania, Philadelphia. Twelve trees of each progeny were examined in a 1952 test planting established by the U.S. Forest Service in Williamstown, Massachusetts. Significant variation in susceptibility to weevil attack has been reported for these and other pedigreed progenies[4].

Leaders or leading lateral shoots of the current season were collected during the winter of 1963 and allowed to dry in the laboratory for one month. This procedure eliminated the problems encountered when working with resinous material. The bark (chiefly cortex) was scraped from the wood cylinder and the woody portion of the stem was further separated into wood and pith. All material was ground in a Wiley mill to pass a 20-mesh sieve.

Conversion of leucoanthocyanin to the coloured aglycone was accomplished by hydrolysing small samples of tissue (100 mg bark and wood, 15 mg pith) in 10 ml. of an *n*-butanol–3 N hydrochloric acid (5 : 1 v/v) solution in a sealed test-tube. This mixture was heated at 100° C in a water bath for 40 min. The transformation of leucoanthocyanins to aglycones may not be quantitative but alcoholic solvents are more than twice as efficient as aqueous solvents[5,6].

After cooling, the coloured extracts were spotted on Whatman No. 1 paper and chromatographed by the ascending method using Forestal solvent (glacial acetic acid–conc. hydrochloric acid–water, 30 : 3 : 10 v/v/v). Pure cyanidin and delphinidin were used as checks. Cyanidin was the only aglycone that could be identified from both parent and progeny trees. Larger samples of bark (up to 3 g) gave similar results.

In order to check further on the earlier report of the occurrence of both cyanidin- and delphinidin-yielding leucoanthocyanins in *P. strobus* bark, four native trees growing within a small area in New Hampshire were sampled. Of these trees, one yielded delphinidin and cyanidin in approximately equal amounts, while another gave no delphinidin. The two other trees were intermediate. Thus it appears that a detectable content of leucodelphinidin is not invariably present in *P. strobus* bark.

Quantitative determinations of cyanidin content in appropriately diluted extracts were made with a Bausch and Lomb 'Spectronic 20' spectrophotometer. This instrument had been calibrated at a wave-length of 550 mμ with known concentrations of cyanidin prepared from pure leucocyanidin (kindly provided by Dr. K. Weinges, Heidelberg) by hydrolysis in butanol–hydrochloric acid. Results, calculated as percentages of dry weight of tissue, are given in Table 1.

There were no significant or meaningful differences in the cyanidin content of bark or wood between trees that had been weeviled or 'unweeviled' in the past. Although there was significant variation among parents in wood cyanidin, the progeny averages were less than might be expected.

Progeny differences in pith cyanidin were highly significant, but again progeny averages were lower than expected and the difference between weeviled and 'unweeviled' trees was not significant. Cyanidin content of the pith was found to decrease with the time since origin. In the single tree tested, pith of the current season had 2·21 per cent cyanidin, while two-year-old and five-year-old pith contained 1·19 per cent and 0·19 per cent cyanidin, respectively.

Several one-year-old stems showed evidence of localized injury by natural causes. When cross-sections of these stems were stained in methanol–hydrochloric acid, it appeared that leucocyanidin had been translocated from the pith through the rays and had accumulated in the wood at the sight of the injury. This movement of leucoanthocyanins in response to injury should be investigated further.

Table 1. Cyanidin Content of Various Tissues of White Pine Leaders

Parent or progeny	Bark (per cent)	Wood (per cent)	Pith (per cent)
G–730	2·54	0·26	3·33
730 × 740	2·59	0·096	1·90
Weeviled	2·54	0·097	1·91
Not weeviled	2·73	0·094	1·86
G–740	2·31	0·08	1·31
730 × 748*	2·64	0·12	1·31
G–748	2·74	0·28	3·71

* All weeviled.

This work was initiated while I was associated with the Northeastern Forest Experiment Station, U.S. Forest Service.

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MICROBIOLOGY

Apparent Suppression of Mutation Rates in Bacteria by Spermine

One of the chief obstacles to chemotherapy with antibiotics has been the frequent emergence of drug-tolerant bacteria. The availability of means to prevent or suppress the development of resistant cultures could have immense medical import as well as providing a valuable tool for the study of mutagenesis. Sevag and collaborators[1-3] reported that when polyamines were included in the culture medium of various micro-organisms, the outgrowth of drug-resistant populations in the presence of low concentrations of antibiotics was significantly delayed. They interpreted these observations as suggesting an antimutagenic role for the polyamines and further proposed that the site for this antimutagenic action is the DNA of the sensitive cells. Because of the theoretical implications of these observations it was of interest to establish in a more rigorous manner whether polyamines are antimutagens or whether the effects described by Sevag and coworkers could be explained in some other manner. This communication presents evidence that the inclusion of spermine in bacterial culture media resulted in a significant decrease in the random mutation rate of *Escherichia coli* and *Staphylococcus aureus*. The mutational events which were investigated were the one-step development of resistance to streptomycin and the back mutation to autotrophic growth by a tryptophan-requiring mutant. Spermine caused even more striking antimutagenic effects when mutations were induced by the inclusion of caffeine in the culture medium[4] or by ultra-violet light. Future papers will seek to demonstrate that bacterial DNA could be the site of action of this compound[5]. A preliminary report on some of these experiments has already been given[6].

E. coli (*UC* 879) and a tryptophan-requiring mutant (*UC* 707) were cultured in a minimal salts glucose medium[7] supplemented with 20 μg/ml. L-tryptophan where required. *S. aureus* (*UC* 76) was cultured on a synthetic medium containing amino-acids, vitamins and salts[8]. To estimate the mutation rates a random fluctuation test, similar to the one described by Luria[9], was used. This test assumes that mutation rates are independent of absolute time and are dependent on the number of bacteria present and the generation time of the cultures[9]. The recent work of Kubitschek and Bendigkeit[10] suggests that this assumption is likely to be warranted in this case. For each treatment 50 replicate 2-ml. cultures were inoculated with 100 cells each and at the end of 18 h of growth at 37° the cultures were plated with nutrient agar containing 800 μg/ml. streptomycin sulphate. When 'back mutation' from tryptophan-requirement was studied, the cultures were twice washed in saline to remove residual tryptophan and plated in glucose salts agar. The number of Petri plates in which no growth occurred was scored and the total number of cells per culture plate was established by plating appropriate dilutions on nutrient agar and counting the number of colonies present. The mutation rate was then calculated from the formula:

$$m = -(2{\cdot}303 \log_{10} P_0)/N$$

where N is the total number of bacteria in a culture and P_0 the proportion of the total cultures which showed no growth.

Table 1 is a summary of a number of experiments in which the random mutation rates to streptomycin resistance by *E. coli* and *S. aureus* were estimated, as well as two experiments in which the 'back mutation' from tryptophan requirement was studied. In all cases the mutation rates of cells which were grown in the presence of 150 μg/ml. spermine·4HCl were clearly lower than those of controls. In most experiments the difference was statistically significant ($P = 0{\cdot}01$–$0{\cdot}007$ as determined from a χ^2 distribution). When spermine was added to control cultures at the time of plating, the mutation rate was the same as that of the controls (line 3, Table 1). Similarly, there was no change in the antimutagenic effect of spermine when the compound was removed along with the tryptophan in the washing procedures and not replaced when the cultures were reconstituted for plating (lines 6 and 7, Table 1). Thus to exert its antimutagenic effect, spermine must be present during the growth of the cells and need not be present during the plating. A reduction of the spermine concentration from 150 μg/ml. to 30 μg/ml. resulted in loss of the antimutagenic effect. However, replacement of the glucose salts medium with nutrient broth in no way influenced the antimutagenic action of spermine (lines 4 and 5, Table 1). Finally, in agreement with the observations of Sevag and Drabble[1], the colonies which grew in the presence of streptomycin were shown on sub-culture to be streptomycin-resistant and those which grew in the absence of tryptophan were shown to have lost their requirement for this amino-acid. Thus, growth in the fluctuation test truly reflected mutational events.

While the suppression of random mutation rate by spermine was real, there was a much more pronounced effect on mutations which were induced by the incorporation of 150 μg/ml. caffeine into the culture medium[4] or by irradiation of the cultures with ultra-violet light. Table 2 presents the results of these experiments. Each experi-

Table 1. Antimutagenic Action of Spermine in Random Fluctuation Tests

Organism	Mutational end-point	Treatment	N $\times 10^9$	M $\times 10^{-10}$	$\frac{m \text{ control}}{m \text{ treated}}$	P
E. coli UC 879	Streptomycin resistance	Control	4·3	1·28	3·7	0·007*
		+ Spermine	4·0	0·35		
E. coli UC 879	Streptomycin resistance	Control; spermine added at plating time	4·3	1·18	1·1	> 0·25†
E. coli UC 879	Streptomycin resistance	Control—broth grown	5·4	1·07	1·95	0·002
		Spermine—broth grown	3·5	0·55		
E. coli UC 707	Trypt$^-$ ⟶ Trypt$^+$	Control	0·76	6·4	2·9	0·026‡
		Spermine	0·83	2·2		
S. aureus UC 76	Streptomycin resistance	Control	6·0	1·09	2·5	0·012‡
		Spermine	5·7	0·43		

The mutation rate, m, was computed from the equation $m = -(2{\cdot}303 \log_{10} P_0)/N$, where N is the total number of bacteria per culture and P_0 the proportion of cultures which were free of mutants. Fifty replicate cultures were used for each treatment. P values are based on χ^2 distribution.
* This experiment is one of 7 similar ones which were performed and is the ranked median of these with respect to magnitude of P. The range in P was 0·17–0·002 and the overall probability of null hypotheses was $\ll 0{\cdot}001$.
† Versus control value, line 1.
‡ One of duplicate experiments with identical results.

Table 2. Antimutagenic Action of Spermine on Mutations to Streptomycin-resistance induced in *E. coli UC* 879 by Caffeine and Ultraviolet Light

(See Table 1 for details)

Treatment	$N_{initial}$ $\times 10^{10}$	$N_{surviving}$ $\times 10^{8}$	$m \times 10^{-10}$	$\frac{m \text{ control}}{m \text{ treated}}$	P
Caffeine	0·8	—	1·5	11·5	⩽0·001
Caffeine + spermine	1·7	—	0·13		
Control	1·5	—	0·48		
Ultra-violet, 25 sec	1·4	0·53	220	8·5	0·024
Spermine + ultra-violet	3·2	0·66	26		
Control	1·4	—	0·46		

ment was repeated once with essentially identical results. The ultra-violet irradiation was accomplished with a Westinghouse 36*GT*5 germicidal lamp at a dose of approximately 150 ergs/mm² to effect a killing rate of approximately 99 per cent[11]. Individual aliquots of a culture which had been grown for 9 h on a shaker (with or without added spermine during growth) were irradiated in a darkened room. All further handling was carried out in the dark to prevent photoreactivation. In all cases cells grown in the presence of spermine had lower mutation rates than the control cells ($P = 0{\cdot}02$).

The mutational event in all these experiments occurred in the absence of the indicator by which the event was scored. Furthermore, there was no effect on the growth rate of streptomycin-sensitive or -resistant organisms when spermine was included in the culture medium. Thus there is no chance that any form of selection could account for the observed changes in the incidence of mutations[12]. Preliminary results suggest that other polyamines may also suppress the random mutation rate much as they were reported by Sevag and co-workers[1–3] to delay the outgrowth of resistant populations in the presence of antibiotics. It is concluded from these results that the antimutagenic action of polyamines is a general property of these compounds, that it is independent of the mutational end-point being measured, and that it is even more pronounced when mutations are induced than when randomly occurring mutations are studied.

We thank Mr. Jack I. Northan for assistance with the statistical analysis of the results.

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VIROLOGY

Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors

It is a well-known fact that certain structurally different chemicals inhibit the synthesis of viral RNA[1–3]. In this communication a few substances which inhibit virus multiplication are described. These are the following: 2-(β-aminoethyl) pyridine (AEP), its substituted derivatives; the 2-pyridyl-ethyl-guanidine sulphate (Pyg)[4], the 2 - (5 - allyl - 3 - methoxy - 2 - hydroxy - benzyl) - β - (α - pyridyl) ethyl amine (*H*-99)[5] and 'Ismelin' 2-octahydro-1-azocynil ethyl guanidine sulphate[4]. Guanidine hydrochloride was used as control.

Experiments were carried out on primary monkey kidney cell monolayer cultured in Petri dishes by the plaque method[6]. The virus inhibitory effect of chemicals was tested against 10–100 plaque-forming units of Sabin's type 1 (*LSc* 2*ab*) virus strain. The plaque assay has already been published in detail[7]. Usually 1 h after virus infection, plates were overlaid with nutrient agar containing 20, 50 or 100 μg/ml. of substances respectively. Sometimes substances were added to the cell sheets, applying a second overlayer 4, 24, 32 h after virus infection respectively. The results are presented in Table 1.

Table 1. Effect of Chemicals on the Plaque-forming Capacity of Type 1 (*LSc* 2*ab*) Virus Strain (Sabin)

Exp. No.	Treatment after virus infection (h)	Concentration of substances (μg/ml.)	Controlled untreated plates	Denomination of substances: 'Ismelin'	Pyg	*H*-99	AEP	Guanidine
				Average number of plaques				
1	1	100	130	Zero	Zero	Zero	N.D.	N.D.
	1			Zero	Zero	Zero	70	
2	4	100	98	Zero	Zero	N.D.*	N.D.	N.D.
	24			39	Zero	N.D.	N.D.	
	1	100		Zero	Zero			
3	1	20		50	4			
	24	100	137	100 (Very small)	Zero	N.D.	N.D.	N.D.
	32	100		N.D.	17			
	1	100		Zero	Zero	Zero		Zero
4	1	50	49	Zero	N.D.	N.D.	N.D.	N.D.
	1	20		Zero	Zero	40		3

*N.D., not done.

As Table 1 indicates, the Pyg was the most effective but its component the AEP had only a slight inhibitory effect. Substance *H*-99 showed a strong inhibition in a concentration of 100 μg/ml., but at 20 μg/ml. its effect was negligible. The inhibitory effect of 'Ismelin' was expressed at any given concentration (100, 50, 20 μg/ml.). When 'Ismelin' was applied 4 h after infection it could inhibit the formation of visible plaques completely, and applying 24 h after infection the plaque number was strongly but not completely inhibited. In the case of Pyg, 4 and 24 h after virus infection the inhibitory effect was complete; moreover, 32 h after infection it was still at about 80 per cent. Consequently these support the hypothesis that 'Ismelin' and Pyg can inhibit the late phase of virus synthesis.

It is remarkable that the highly active chemicals are known as anti-hypertensive substances[8], and one of them, 'Ismelin', is already being used as a therapeutic drug.

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GENETICS

Puffing of Salivary Gland Chromosomes after Treatment with Carbon Dioxide

After larvae of the Oregon *R* strain of *Drosophila melanogaster* had been placed in an atmosphere of CO_2 for 1 h late in the third instar, the salivary-gland chromosomes were examined and compared with those of a similar group of the same age. The chromosomal regions

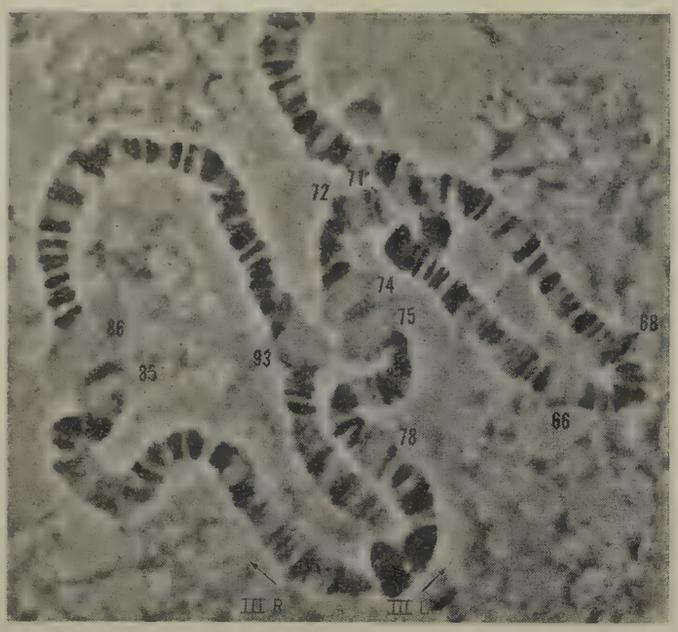

Fig. 1

studied were eight regions known to puff: 85–86, 93, and 95 on the right arm of the third chromosome; 66, 71–72, 74–75, and 78 on the left arm of the same chromosome; and 2*B* on the *X* chromosome. The size of the puffs in the 84 glands examined were arbitrarily divided into four classes from smallest to largest and the data tabulated accordingly (Table 1). Although not all puffs were larger in the groups treated, the mean size of puffs III *R* 85–86, 93, and 95, and III *L* 66 was larger after exposure to CO_2. The remainder showed no tendency to enlarge after such treatment. Larvae late in the third instar not used for chromosomal studies survived and developed into adults following the exposure to CO_2. Many younger larvae died. These observations are reported because of current interest in the puffing pattern as presumed indication of localized production of RNA in response to hormonal stimulation. Recently we have found that ecdysone will produce puffing at this stage of larval development in some regions but not in others. Two not affected by CO_2 (I 2*B* and III *L* 74–75) showed an increase in mean size with ecdysone, and one (III *L* 71–72) failed to respond to either treatment.

Table 1. Effect of CO_2 on Size of Puffs examined in Chromosomes I and III

Chromosome	Size of puff	Control Number	Control Percentage	Experimental Number	Experimental Percentage
III *R*–85, 86*	1	213	91	26	16
	2			14	9
	3	9	4	24	15
	4	15	6	96	60
III *R*–93*	1	168	72	27	17
	2	51	22	28	17
	3	12	5	55	34
	4	1	1	50	31
III *R*–95*	1	213	92	62	39
	2	19	8	95	59
	3			3	2
	4				
III *L*–66*	1	218	94	48	31
	2	11	5	70	44
	3	2	1	27	17
	4			12	8
III *L*–71, 72	1	88	37	86	54
	2	148	62	74	46
	3	1	1		
	4				
III *L*–74, 75	1	41	18	35	22
	2	137	59	81	50
	3	34	14	25	16
	4	20	9	19	12
III *L*–78	1	199	86	156	97
	2	28	12	3	2
	3	6	2	1	1
	4				
I–2*B*	1	48	21	50	31
	2	161	74	107	67
	3	22	10	1	1
	4	1	1	2	1

* Mean size increased by CO_2.

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Evidence for a Second 'Structural' Locus determining Human Phosphoglucomutase

At least seven isoenzyme components[1] of human phosphoglucomutase (PGM) can be distinguished electrophoretically (*a*–*g*, Fig. 1). Individual differences occur in the pattern of components present, and three commonly occurring phenotypes have been recognized (PGM 1, PGM 2–1 and PGM 2). They differ in the distribution of components *a*, *b*, *c* and *d*. Components *a* and *c* are present in PGM 1 and PGM 2–1, while components *b* and *d* are present in PGM 2–1 and PGM 2. The genetical evidence indicates that these phenotypic differences are due to a pair of autosomal alleles (PGM^1 and PGM^2). Evidently a structural locus is involved, and it seems likely that one of these alleles determines the formation of a polypeptide common to *a* and *c*, while the other allele determines the formation of an alternative polypeptide common to *b* and *d*. The three phenotypes do not differ from one another in the occurrence of components *e*, *f* and *g*. This suggests that at least one other 'structural' locus is involved in the determination of these latter components.

Evidence for the existence of such a locus has now been obtained from the investigation of a family in which two unusual PGM phenotypes were found to be segregating. These new phenotypes are illustrated diagrammatically in Fig. 1. They will be referred to for the present as PGM 1 (Atkinson) and PGM 2–1 (Atkinson), after the name of the family in which they occurred. The peculiar features of the 'Atkinson' phenotypes involve the rapidly migrating (*efg*) set of components. Usually components *e* and *f* are very much more intense than component *g*. In the 'Atkinson' phenotypes components *e* and *f*, though present,

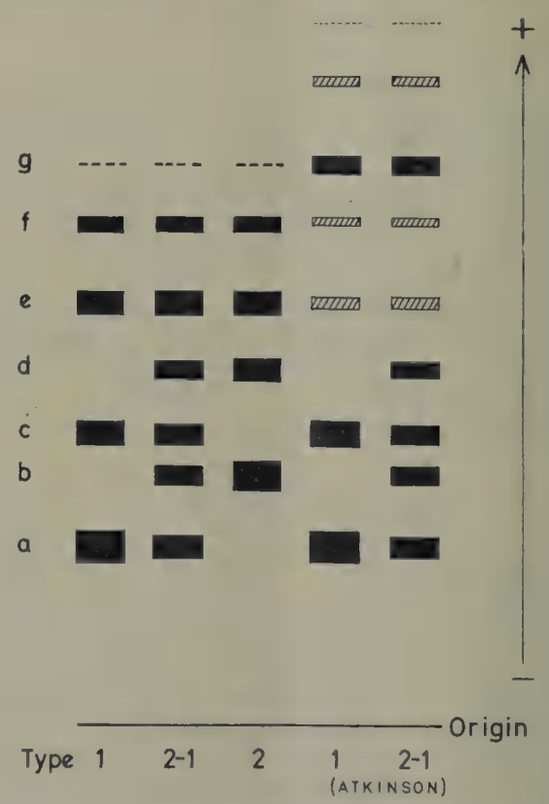

Fig. 1. Diagram of starch gel showing PGM components observed in haemolysates from individuals of the common phenotypes 1, 2–1 and 2, and of the rare phenotypes 1 (Atkinson) and 2–1 (Atkinson). The electrophoretic and staining techniques are described in ref. 1

are relatively weak, but there is an intense component with a mobility similar to *g*, and a further intense component with a mobility greater than *g*. An even faster moving but extremely weak component is also probably characteristic of these phenotypes. This pattern of components was observed in three members of the family (Fig. 2). In one of them (I_1) it was associated with *a* and *c* components typical of PGM 1. In the others (II_1 and III_2) it occurred together with components *a*, *b*, *c* and *d* which are characteristic of PGM 2–1.

Since the unusual pattern of fast moving components was observed in individuals from three successive generations of the same family, it seems likely that it is determined by an uncommon gene for which these individuals were heterozygous. This gene evidently segregates independently of the genes previously designated PGM^1 and PGM^2 since it appears to have been transmitted by II_1 to one but not the other of his non-identical twin daughters, both of whom had the *a*, *b*, *c* and *d* components typical of PGM 2–1, and must have received the PGM^2 gene from their mother.

The electrophoretic patterns characteristic of the 'Atkinson' phenotypes suggest that the unusual gene which is present causes a specific structural alteration in components *e*, *f* and probably also in *g*. Individuals I_1, II_1 and III_2 are presumably heterozygous for this gene and its common allele, and the observed patterns can be most simply explained if they are considered as arising from a mixture of components *e*, *f* and *g* determined by the normal allele present, and the altered counterparts of these components determined by the unusual allele. If the structural alteration causes a net decrease in positive charge, such that the altered component *e* now has a mobility similar to that of the usual component *g*, and the alteration to components *f* and *g* results in a similar change in net charge, then the observed pattern can be accounted for.

The pedigree and electrophoretic findings suggest that genes at two distinct and not closely linked loci both of which affect the structure of phosphoglucomutase components are segregating in this family. We may designate the locus previously recognized as specifically affecting components *a*, *b*, *c* and *d* as PGM_1, and the locus which

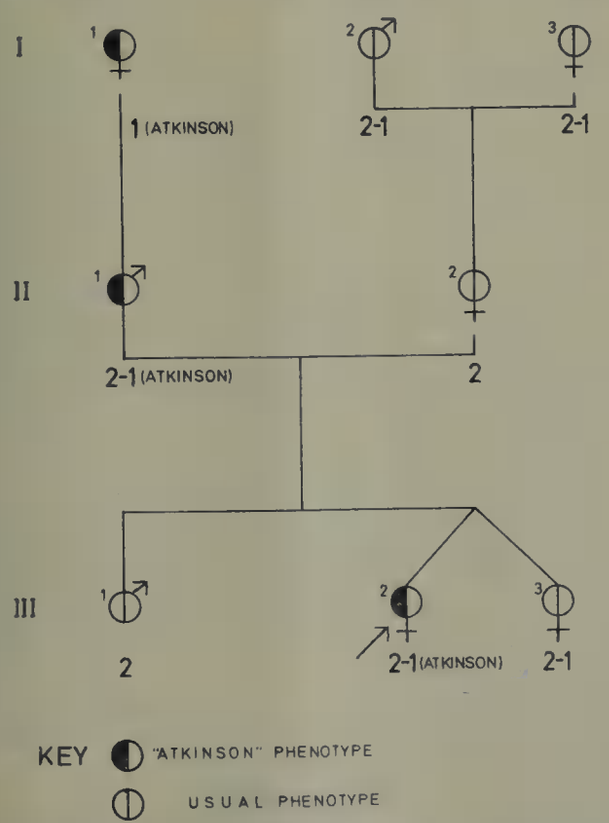

Fig. 2. Pedigree of family (*MRC* 225) showing segregation of PGM phenotypes

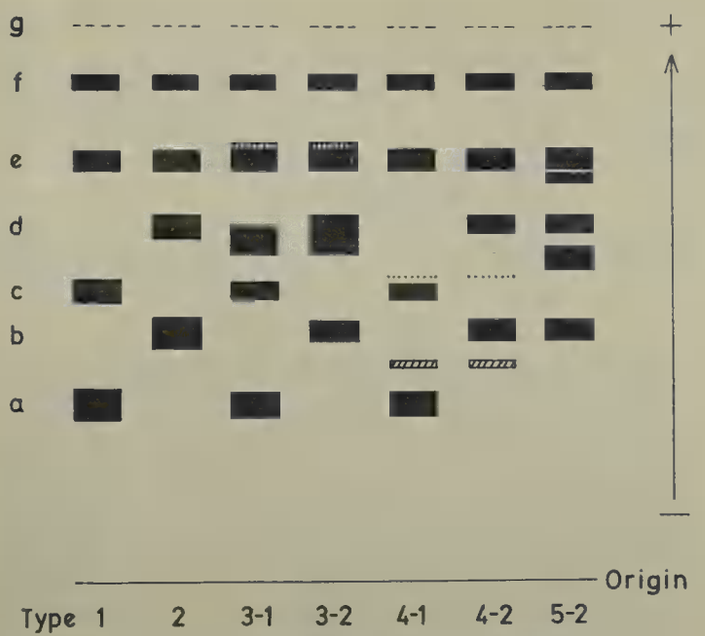

Fig. 3. Diagram of starch gel showing PGM components observed in haemolysates from individuals of the common phenotypes 1 and 2, and the rare phenotypes 3–1, 3–2, 4–1, 4–2 and 5–2

specifically affects components *e*, *f* and *g* as PGM_2. The common allele at PGM_2 for which most individuals are homozygous may then be designated PGM_2^1, and the uncommon allele present in individuals with the 'Atkinson' phenotypes PGM_2^2. In these terms the genotype postulated for the phenotype PGM 1 (Atkinson) is PGM_1^1-$PGM_1^1/PGM_2^1PGM_2^2$, and the genotype corresponding to PGM 2–1 (Atkinson) is $PGM_1^1PGM_1^2/PGM_2^1PGM_2^2$.

In the course of family and population investigations in which PGM in more than 2,000 blood samples from different individuals has been examined, several further uncommon phenotypes have been identified. Five of these are illustrated diagrammatically in Fig. 3. In one family eight individuals with phenotype PGM 3–1 and two individuals with PGM 3–2 were observed. The pedigree suggested that these phenotypes represent heterozygotes for an uncommon allele PGM_1^3 with PGM_1^1 and PGM_1^2, respectively. The allele PGM_1^3 probably determines the formation of two new components one of which has a mobility slightly slower than *d*, and the other slightly faster than *e*. PGM 4–1 and PGM 4–2 were also encountered among a group of related individuals. There were in all six individuals with phenotype PGM 4–1 and two with PGM 4–2, and the segregation pattern suggested that they were heterozygotes for a further allele PGM_1^4 with the common alleles PGM_1^1 and PGM_1^2, respectively. PGM_1^4 probably determines the formation of two components, one of which has a mobility intermediate between that of *a* and *b*, and the other a mobility slightly greater than *c*. Both these new components and particularly the faster one were very weak by comparison with the *a* and *c* or the *b* and *d* components with which they occurred. It seems possible, then, that the rare homozygotes $PGM_1^4PGM_1^4$ when identified will exhibit a marked reduction in level of PGM activity. The phenotype PGM 5–2 has so far been identified in only one individual and family data are not available. The PGM of this individual showed two new components, one of which had a mobility intermediate between *c* and *d*, and the other a mobility intermediate between *d* and *e*. Components *b* and *d* were also present, and by analogy it was considered likely that the two new components were determined by an uncommon allele PGM_1^5 which in this individual occurred in heterozygous combination with PGM_1^2. In each of the new phenotypes PGM 3–1, PGM 3–2, PGM 4–1, PGM 4–2 and PGM 5–2, the *e*, *f* and *g* components were apparently not affected and one may suppose that the individuals concerned were all homozygous for PGM_2^1. Full details of these family investigations will be published elsewhere.

We are grateful to Dr. J. H. Edwards for sending us samples from the family in Fig. 2.

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ANTHROPOLOGY

Hair Colour of the Population of Tristan da Cunha

As a result of the volcanic eruption on Tristan da Cunha in 1962 some 268 islanders were evacuated to the United Kingdom. During their stay in this country many of them agreed to co-operate in a number of investigations concerning their physical condition and characteristics organized by the Medical Research Council. Their pigmentation was examined as part of this programme; I was concerned with hair colour.

People the world over show a certain reluctance to part with quantities of their head hair sufficient for detailed laboratory investigation of pigmentation. In this respect the Tristanites were no exception, and only some 131 individuals provided useful quantities of hair.

A number of investigations of hair pigmentation including those of Reed[1], Sunderland[2] and Barnicot[3] have utilized spectrophotometric data including, in particular, percentage reflectance values, as means of denoting brightness and hue. These procedures involving precise quantified information introduced a measure of objectivity into such studies which had been less evident in previous work. Thus Reed[1], for example, denoted redness by the R statistic with $R = 100(y_{530}-0{\cdot}243y_{400})/Y$ 650) while Sunderland[2] used size (Q), shape (P) and both combined (L) statistics in order to characterize the percentage reflectance curves for the hair samples which he studied.

It was hoped that sufficient hair would have been available from the Tristanites to allow the measurement of percentage reflectance values and subsequent statistical analysis of the data, as was possible in the enumerated studies. However, the quantities of hair available disallowed the use of spectrophotometric techniques. The only, fairly satisfactory, alternative means of characterizing this pigmentary variable appeared to be the use of the *Haarfarbentafel nach Fischer-Saller*. This consists of thirty tufts of human hair ranging in colour from very blond (Weiss blond), designated by the letter A at one extreme, to the darkest shades of hair (*Braun schwarz*), designated by the letters U–Y at the other, together with the intermediate categories *Hell blond* (B–E), *Blond* (F–L), *Dunkel blond* (M–O) and *Braun* (P–T) in order of increasing darkness. The Roman numerals I–IV cover a range of red hair shades; V and VI the red-blond range. Hair specimens are matched with these standards and the colours characterized accordingly. In this investigation, the matching was carried out independently by two people, working in good daylight. Their agreed results are summarized in Table 1.

None of the lighter shades of hair colour (A–L) is represented in this population, and only three individuals have hair as light as the *Dunkel blond* category. In fact, the great majority of the islanders have dark brown–black hair, the only dramatic exception being the unique vividly red-haired individual. Traces of red hair, usually single red hairs among predominantly brown–black hairs, are occasionally visible in this population.

Table 2 shows the relationship between hair colour and sex.

There do not appear to be any significant sex differences. Therefore, taking both sexes together, Table 3 shows the relationship between hair colour and age.

Table 1. Fischer-Saller Hair Colour Designations for the Tristan da Cunha Sample

Fischer-Saller designation	Number of specimens		Fischer-Saller category
M	1	3	
N	1	2·29%	*Dunkel blond*
O	1		
P	0		
Q	1	9	
R	0	6·87%	*Braun*
S	4		
T	4		
U	8		
V	9		
W	13	118	*Braun schwarz*
X	46	90·08%	
Y	42		
II	1	1	
		0·76%	*Rot*
Total	131	100·00%	

Table 2. Hair Colour and Sex

Fischer-Saller category	Males (No.)	Males (% of total)	Females (No.)	Females (% of total)
Dunkel blond	2	3·12	1	1·49
Braun	3	4·69	6	8·96
Braun schwarz	59	92·19	59	88·06
Rot	0	0·00	1	1·49
Total	64	100·00	67	100·00

Table 3. Hair Colour and Age

Age group (years)	*Dunkel blond* No.	*Dunkel blond* % Total	*Braun* No.	*Braun* % Total	*Braun schwarz* No.	*Braun schwarz* % Total	*Rot* No.	*Rot* % Total	Total
0–15	2	4·3	7	15·2	36	78·3	1	2·2	46
16–30			1	3·3	29	96·7			30
31–45					26	100·0			26
46–60					15	100·0			15
61–81					8	100·0			8
	2	1·6	8	6·4	114	91·2	1	0·8	125

It is well known from a number of investigations, including those of Cowie and Penrose[4] and Sunderland[2], that hair colour darkens with age. In the present results, it is interesting to note that the fairest people, in the *Dunkel blond* category, are in the youngest age bracket (0–15 years) and that the *Braun* category likewise is found only among individuals aged 30 years and less. Again, red hair fades with increasing age. This was clearly demonstrated by Reed[1] and by Sunderland[2]. Among red-haired individuals, the R statistic increases in value with the years; that is, their red hair becomes less so. Here, the only red-haired individual is in the 0–15 age category, and it may well be that at a more advanced age this individual's hair may become much less red or even predominantly brown. All individuals aged 31 years and over have *Braun schwarz* hair.

Quite evidently, this population is predominantly darkly pigmented. Some of the early settlers on Tristan were north-west Europeans, including Glass, Swain and Green, while Rogers and Hagan were American. However, others were from predominantly darker populations, including Repetto and Lavarello from Italy, five women from St. Helena who arrived on the island in 1827 and who are reported to have been of mixed European and 'coloured' parentage and also William Glass's wife from the Cape Coloured population of South Africa. These origins summarized by Harris *et al.*[5] and the subsequent hybridization on Tristan adequately account for the hair pigmentation characteristics of the islanders.

Any estimate of the quantitative contributions of the Europeans, Americans, Italians, St. Helena people and Cape Coloureds (and possibly other groups also) to this population is fraught with difficulty. So far, even the initial step of comparing the hair colour of the Tristanites with the populations listed is impossible since hair colour data, particularly using the *Haarfarbentafel nach Fischer-Saller*, is not available for those groups.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 25

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Symposium on "The Changing Face of Reinforced Plastics".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. E. B. Atkinson: "Flow Behaviour of Molten Polymers".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. E. G. Bowen: "The Welsh Colony in Patagonia".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. F. W. Gibbs: "Gunpowder Priestley and Dictionary Johnson".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Sir John Cockcroft, O.M., F.R.S.: "Competitive Nuclear Power".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.–I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. W. J. Poppelbaum: "Opto-Electronics".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. J. T. Bull: "A.C. Traction Power Supplies" (Chairman's Address).

Tuesday, October 26

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10 a.m.—Meeting on "Land Restoration".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Levels of Being: What is Man?" *

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. J. V. Bartlett, Mr. T. M. Noskiewicz and Mr. J. A. Ramsay: "Soft Ground Tunnelling for the Toronto Subway".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Emission from Conductors into Dielectrics" opened by Dr. T. J. Lewis.

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. H. J. B. Atkins: "Charles Darwin and His Influence" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. S. V. Burgen: "The Salivary Glands". (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Make Your Workshop Buzz with a Machine Loading Computer Programme".

Wednesday, October 27

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 4.30 p.m.—Annual General Meeting. Prof. P. M. S. Blackett, C.H., F.R.S.: "The Continental Drift" (Duke of Edinburgh's Lecture).

INSTITUTE OF FUEL (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5.30 p.m.—Prof. G. J. Hills: "The Electrochemistry of Deposits of Inorganic Constituents of Fuels at High Temperatures".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. H. Norman G. Allen, C.B.E.: "Ex Opera et Industria" (Presidential Address).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. J. Yudkin: "Appetite Control".

Thursday, October 28

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. C. Vita-Finzi: "The Mediterranean During the Ice Age".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. Monica M. Cole: "Biogeography in the Service of Man (with particular reference to the Underdeveloped Lands)" (Inaugural Lecture).*

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. B. Bentham: "The History of Exploration and Development of Oil in Burma".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Eleanor Zaimis: "Immunological Sympathectomy". (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Temperature Limits of Liquid Lubricants".

TELEVISION SOCIETY (joint meeting with the Institution of Electrical Engineers and the Institution of Electronic and Radio Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 6 p.m.—Dr. W. Bruch: "The P.A.L. Colour Television System".

Friday, October 29

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at the Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 4.30 p.m.—Meeting on "Microchemistry—Past, Present and Future". Speakers: Prof. R. Belcher, Mr. C. Whalley and Prof. C. L. Wilson.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. H. H. Watson: "Some Recent Developments in Ultra-High-Vacuum Technology".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Baroness Jane van Lawick-Goodall: "Infancy, Childhood and Adolescence in the Wild Chimpanzee".

Saturday, October 30

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. Robinson: "Natural History of a Landslide".*

Monday, November 1

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 9.30 a.m. and 2.15 p.m.—Colloquium on "HS 303 (Early Bird) and the Post Office Earth Station at Goonhilly".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. R. Reid: "Ageing".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. D. A. Price Evans: "Genetics and Drug Idiosyncrasy".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. I. Scrub-itch and the Ecologist".*

UNIVERSITY OF LONDON (in the Chemistry Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. J. Millen: "Molecular Measurements" (Inaugural Lecture).*

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "The Doctor's View of Engineering: The Engineer's View of Medicine".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Island" (film with introduction by Prof. C. A. Fisher).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dame Kathleen Lonsdale, D.B.E., F.R.S.: "Crystallography as a Research Tool in Chemistry" (Jubilee Memorial Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

INSPECTOR GRADE III (Scientific Officer, not over 35, with a recognized first- or second-class honours university degree in which zoology was taken as a major subject) IN THE DEPARTMENT OF LANDS, Dublin, for work in connexion with the development of game resources—The Secretary (S.18), Civil Service Commission, 45 Upper O'Connell Street, Dublin, 1, Republic of Ireland (October 26).

SENIOR SCIENTIFIC OFFICER or SCIENTIFIC OFFICER (with a Ph.D., or good honours degree with equivalent research experience in genetics or plant breeding) for breeding work and study of breeding methods with vegetables—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 29).

CHAIR OF ORGANIC CHEMISTRY—The Registrar, University of Kent at Canterbury, Canterbury, Kent (October 30).

LECTURER or ASSISTANT LECTURER IN SOCIOLOGY—The Secretary, The University, Aberdeen (October 30).

LECTURERS IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne, 2 (November 1).

TUTORIAL STUDENT IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, King's College, Strand, London, W.C.2 (November 1).

BIOCHEMIST (with an honours degree in biochemistry and some research experience or a Ph.D.) IN THE PSYCHOSOMATIC RESEARCH UNIT, DEPARTMENT OF PHYSIOLOGICAL MEDICINE (University of Glasgow), to assist with the investigation of aspects of endocrine function in certain bodily illnesses in human subjects—The Director of the Psychosomatic Research Unit, Southern General Hospital, Glasgow, S.W.1 (November 5).

LECTURER IN THE DEPARTMENT OF ZOOLOGY, University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 10).

LECTURER (with an honours degree in electrical engineering) IN ELECTRICAL ENGINEERING at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 10).

READER IN IMMUNOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 10).

LECTURER/ASSISTANT LECTURER (with at least a good honours degree with suitable teaching and research experience, and preferably specialized in some branch of solid state physics) IN THE DEPARTMENT OF PHYSICS, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, November 15).

LECTURER or ASSISTANT LECTURER IN GEOGRAPHY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 15).

LECTURER (with a veterinary qualification) IN VETERINARY MEDICINE—The Secretary, Trinity College, Dublin, 2, Republic of Ireland (November 15).

LECTURER (preferably with research interests in astrophysics, optical astronomy, radio astronomy or plasma physics) IN PHYSICS at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 19).

SENIOR LECTURER (preferably with registrable veterinary qualifications) IN VETERINARY PHYSIOLOGY IN THE DEPARTMENT OF VETERINARY PRE-CLINICAL SCIENCES, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 26).

DIRECTOR OF THE MARINE BIOLOGICAL LABORATORY AT MILLPORT, Scottish Marine Biological Association—Dr. C. M. Yonge, C.B.E., F.R.S., 13 Cumin Place, Edinburgh, 9 (November 30).

SENIOR LECTURER or LECTURER (with a degree in mechanical engineering, preferably at honours level, of a recognized Commonwealth University, together with either experience in industry or research and teaching experience at university level) IN MECHANICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

LECTURER or SENIOR LECTURER IN CHEMICAL PATHOLOGY, University of Otago Medical School, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 15).

CHAIR OF PHYSIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 4).

AGRICULTURAL CHEMIST (national of the United Kingdom or the Republic of Ireland, with a second-class honours degree in agricultural chemistry or chemistry, plus at least two years' postgraduate training or experience in soil science) in Uganda, for research work on soil fertility problems connected with tropical rotations, manures and fertilizers—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/022.

ASSISTANT CONSERVATOR OF FORESTS (national of the United Kingdom or the Republic of Ireland, under 40, with a degree in forestry) in Kenya, for work in connexion with the administration and technical direction of a forest division—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 324/95/01.

BIOLOGIST (national of the United Kingdom or the Republic of Ireland, between 24 and 35 years, with an honours degree in zoology and postgraduate experience in animal ecology) in Tanzania, to investigate the ecology of game animals in the woodland areas—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 275/173/01.

BIOLOGIST (preferably with a Ph.D. or medically qualified, and interests or experience in the problem of graft versus host reaction, immunological tolerance and mitotic synchronization) for a research post in a unit working on trophoblastic tumours and leukaemia in the Charing Cross Hospital Group—Dr. K. D. Bagshawe, Edgar Laboratory, Fulham Hospital, London, W.6.

CHEMICAL or BIOCHEMICAL TECHNICIAN to assist in research on muscle disease—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1.

DEMONSTRATORS (2) IN THE DEPARTMENT OF PHYSICS for laboratory demonstrating work and assistance with student tutorials—The Registrar, University College of Wales, Aberystwyth.

LECTURER or ASSISTANT LECTURER IN THEORETICAL PHYSICS—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

LECTURER (well qualified academically with postgraduate experience in industry and/or teaching) IN PHYSICAL CHEMISTRY to teach to B.Sc. and Grad.R.I.C. Part II level—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

OIL PALM AGRONOMIST (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture and postgraduate training, preferably with previous experience as an agronomist) in Sarawak, to open up and establish a new experimental station, mainly concerned with oil palm and other crops new to Sarawak, for example, cocoa and hemp—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/155/08.

RESEARCH ASSISTANT or RESEARCH STUDENT to work in the Department of Physics with a small group investigating the application of existing and novel techniques of geophysical prospecting to archaeology—The Registrar, Ref. 126Y/E, Bradford Institute of Technology, Bradford, 7.

RESEARCH FELLOW (with a higher degree and preferably experience in the field of semiconductor physics, technology or chemistry) IN THE SCHOOL OF PHYSICS, to work on new direct-gap semiconductor compounds for use as semiconductor lamps, lasers and Gunn devices—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 65/98.

RESEARCH OFFICER (Virologist) (national of the United Kingdom or the Republic of Ireland, with an honours degree in botany or agricultural botany and experience in plant virology) with the East African Common Services Organization, to study virus diseases affecting agricultural crops and to help with the scientific supervision of the East African Plant Quarantine Station—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting RC 213/214/011.

SENIOR LECTURER IN SOCIAL ANTHROPOLOGY; a SENIOR LECTURER or LECTURER IN PHILOSOPHY; a LECTURER IN PSYCHOLOGY; and a LECTURER IN ENTOMOLOGY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

SOIL CONSERVATION ENGINEER (national of the United Kingdom or the Republic of Ireland, with a second-class degree in agricultural engineering, or a degree in soil conservation, or a degree in civil engineering and soil conservation, and preferably experience in soil conservation) in Uganda, to carry out soil conservation measures on projects, to check layouts, to train field staff and to collect data and prepare a handbook—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/020.

SOIL SURVEY OFFICER (national of the United Kingdom or the Republic of Ireland, with an honours degree in either natural science, agriculture or geography, with, if possible, postgraduate training or experience in soil science) in Uganda, to carry out soil surveys as a member of a land planting team working on the development of group farms—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/019.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Time Units in the Solar System—Summary. By S. Ryzewski. Pp. 23. (Southampton: S. Ryzewski, 15 Landguard Road, 1965.) [149

British Medical Bulletin, Vol. 21, No. 3 (September, 1965): Symposium on Recent Research in Molecular Biology. Pp. 183–278. (London: The British Council, 1965.) 30*s.* [149

Surface Active Agents: Their Extraction, Characterisation and Determination. By Maurice Bell. Pp. 24. (Leeds: Glovers (Chemicals), Ltd., 1965.) 5*s.* [149

Loughton College of Further Education. Prospectus for Day and Evening Classes, 1965–1966. Pp. 48. (Loughton: Loughton College of Further Education, 1965.) [149

Department of Education and Science. Chemicals, Additives and Drugs—a Short Working Guide to the Structure of Government Committees and Panels. Pp. 12. (London: Department of Education and Science, 1965.) [149

Bulletin of the British Museum (Natural History). Geology, Vol. 11, No. 2: Some Lower Cretaceous Terebratelloidea. By Ellis Frederic Owen. Pp. 47–72+3 plates. (London: British Museum (Natural History), 1965.) 20*s.* [149

The Role of Chemical Engineering in Process Development. By Dr. G. H. Reman. (Third Marchon Lecture delivered 6th November 1964 at the University of Newcastle upon Tyne.) Pp. 35. (Newcastle upon Tyne: The University, 1965.) 6*s.* 6*d.* including postage. [149

City of Liverpool College of Technology. Prospectus of Part-Time Courses, Session 1965–1966. Pp. 63. (Liverpool: City of Liverpool College of Technology, 1965.) [149

The Natural Rubber Producers' Research Association. Twenty-seventh Annual Report. Pp. 43. (London: The Natural Rubber Producers' Research Association, 1965.) [149

Department of Education and Science. National Lending Library for Science and Technology. Current Serials Received by the N.L.L., March 1965. Pp. xii+322. (London: H.M. Stationery Office, 1965.) 27*s.* 6*d.* net [149

Other Countries

Geological Survey of New South Wales. Report No. 17: Quality Classification of Water. Compiled by R. J. Griffin. Pp. 10. Report No. 20: Evan's Head Town Water Supply. By R. J. Griffin. Iluka Town Water Supply. By R. J. Griffin. Bowning Town Water Supply. By R. J. Griffin. Tumbarumba Town Water Supply. By J. Ringis and P. McEwen. Pp. 26. (Sydney: Department of Mines, 1965.) [149

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Scientific Reports, Series B (II) Botany. Publication No. 78: Observations on the Fungi of Macquarie Island. By J. S. Bunt. Pp. 22. (Melbourne: Antarctic Division, Department of External Affairs, 1965.) [149

Tables Générales des Mémoires de l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1772–1897. Pp. 260. (Bruxelles: l'Académie Royal des Sciences, des Lettres et des Beaux-Arts de Belgique, 1964.) [149

Rubber Research Institute of Malaya. R.R.I.M. Planting Manual No. 11: Clones of *Hevea brasiliensis* of Commercial Interest in Malaya—a Summary of Available Information on the Parentage, Distribution, Yields, and Secondary Characteristics of Clones Planted Commercially or Showing Promise in Trials. By C. C. Paardekooper. Pp. 135. (Kuala Lumpur: Rubber Research Institute of Malaya, 1965.) 3 Malayan dollars. [149

India: Council of Scientific and Industrial Research. Survey and Planning of Scientific Research Unit. Survey Report No. 5: Research Efforts in Industrial Establishments in India. By S. H. M. Husaini, A. Ghosal and A. Rahman. Pp. ii+42. (New Delhi: Council of Scientific and Industrial Research, 1965.) [149

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tribophysics, 1964–65. Pp. 18. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [149

India: Council of Scientific and Industrial Research. Survey and Planning of Scientific Research Unit. Science in India. Compiled by Mohan Bhatia. Pp. ii+67. (New Delhi: Council of Scientific and Industrial Research, 1965.) [149

Annual Report of the Geological Survey, Borneo Region, Malayasia, for 1964. By R. A. M. Wilson. Pp. vii+222+60 plates. (Kuching, Sarawak: Geological Survey Department, Borneo Region, Malayasia, 1965.) 3 Malayan dollars; 7*s.* [149

Université Leopoldville. "Zooleo" No. 1: Quel est ce Termite Africain? Par A. Bouillon et G. Mathot. Pp. 64+25 planches. 120 francs; 17*s.* 2*d.* "Zooleo" No. 2: Serpents Danger ! Par A. Bouillon. Pp. 76. 80 francs; 11*s.* 6*d.* (Bruxelles: Termites Africains, 33 rue Berckmans, 1965.) [149

Australia: Commonwealth Scientific and Industrial Research Organization. Mineragraphic Investigations—Annual Report 1964–65. Pp. 14. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [149

Colony of Mauritius. Meteorological Observations and Climatological Summaries. April, 1964. Pp. 37. May, 1964. Pp. 37. June, 1964. Pp. 38. (Port Louis: Government Printer, 1965.) [149

India: Council of Scientific and Industrial Research. Annual Report of the Central Building Research Institute, 1964–65. Pp. ix+83. (Roorkee (U.P.): Central Building Research Institute, 1965.) [149

The Resources Agency of California: Department of Fish and Game. Fish Bulletin No. 127: California "Catchable" Trout Fisheries. By Robert L. Butler and David P. Borgeson. Pp. 47. Fish Bulletin No. 128: An Analysis of California's Albacore Fishery. By Harold B. Clemens and William L. Craig. Pp. 301. (Sacramento, California: Office of Procurement, Documents Section, P.O. Box 1612, 1965.) [149

Seminaire sur la Protection Contre les Dangers du Tritium, Le Vesinet, 16–18 Avril 1964. (Section Française de la Health Physics Society. Service Central de Protection Contre les Rayonnements Ionisants. Société Europeenne de Protection Contre les Rayonnements.) Pp. vi+136. (Le Vesinet (S. et O.): Service Central de Protection Contre les Rayonnements Ionisants, 1965.) [149

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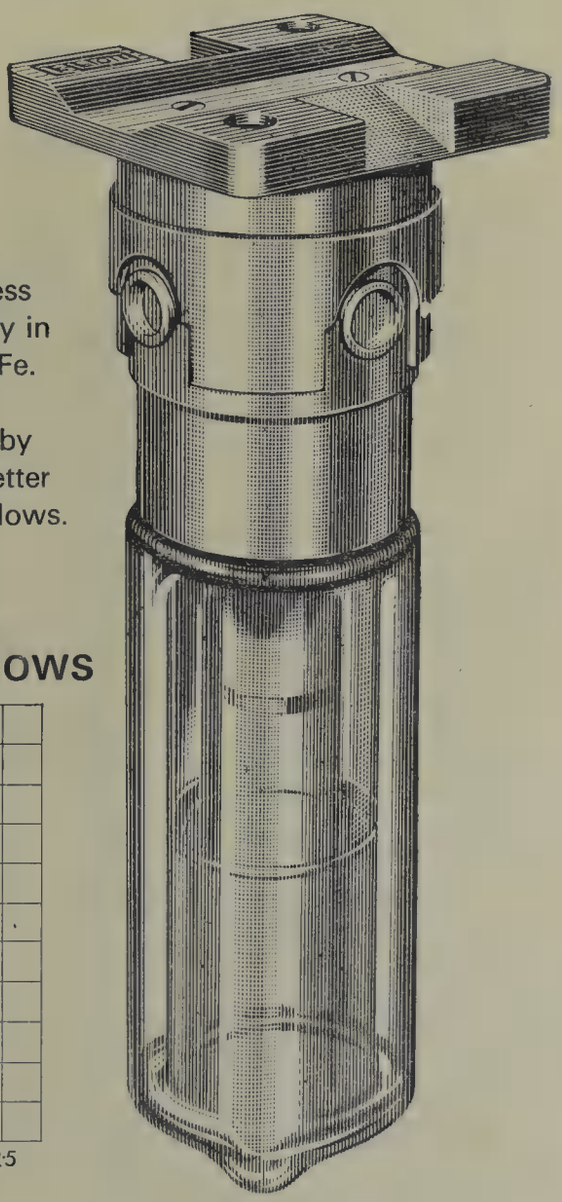

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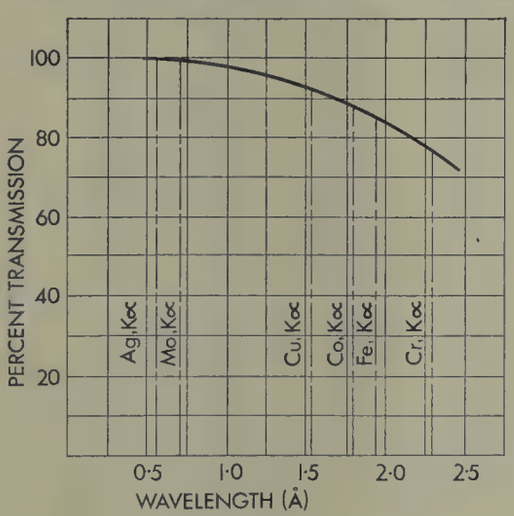

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Applications should reach Dr. Ralph F. Voigt, Head of Department of Pharmacognosy and Pharmacology by November 15, 1965.

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Applications are invited for appointment as Lecturer/Assistant Lecturer in the Department of Physics. Candidates should have at least a good honours degree with suitable teaching and research experience. Preference will be given to applicants specialized in some branch of Solid State Physics.

Salary scale: The amounts mentioned below refer to Malayan dollars (per mensem) and their approximate sterling equivalents (per annum), are shown in parentheses. Lecturer: $920 by $40 to $1,160/$1,200 by $45 to $1,425 (£1,338 by £56 to £1,624/£1,680 by £63 to £1,995); Assistant Lecturer: $800 by $40 $880 (£1,120 by £56 to £1,232). In addition the following allowances are payable: Variable allowance: 35 per cent of basic salary, subject to certain maxima depending on marital status, minimum $150 per mensem (£210 per annum), maximum $400 per mensem (£560 per annum). Inducement allowance (if awarded), varies: (a) For Lecturers, from $0 to $300 per mensem (£420 per annum); (b) For Assistant Lecturers, from $0 to $200 per mensem (£280 per annum). Housing will be provided, if available, for which a rent of $100 to $125 per mensem (£117 to £175 per annum) will be charged. Provident Fund: Staff members contribute 5 per cent and University 15 per cent of basic salary.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. The closing date for the receipt of applications in Kuala Lumpur and London is November 15, 1965.

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TEMPORARY LECTURESHIP IN ZOOLOGY

Applications are invited for an appointment as temporary Lecturer in the Department of Zoology for a period of up to three years from January 1, 1966. The salary range for the position is £A2,400 by £A110 to £A3,170 per annum. A travelling allowance will also be granted. The appointee will be required to assist with the teaching of first-year classes and with the supervision of postgraduate students working on the physiology and ecology of marsupials and other vertebrates. However, applications from persons with other interests will be considered and the head of the Department (Professor H. Waring) will welcome inquiries from prospective candidates. The appointee will be encouraged to undertake personal research. In addition to good research facilities on the campus for physiological, ecological and marine studies, there are two field stations and motor transport.

Intending applicants are requested to obtain details of the procedure to be followed in applying for the post and a copy of the conditions of appointment before submitting their applications. This information is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on December 4, 1965.

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BIOMETRICIANS

Applications invited for pensionable posts in the Biometrics Branch of the Ministry of Agriculture. Duties will be mainly concerned with the design and analysis of laboratory and field experiments and of surveys and with the statistical assessment of genetical studies on poultry, cattle and herbage plants. Time on the new Government computer will be available to the Branch. Qualifications, first- or second-class Honours degree or the equivalent in Mathematics or Biology with specialization in and/or experience of statistics, with special reference to the design and analysis of experiments and surveys. Salary scales, £895 to £1,521; £1,685 to £2,082. Scale and initial salary offered will depend on qualifications and experience. Preference for ex-Service candidates.

Application forms obtainable from the Secretary, Civil Service Commission, Stormont, Belfast, 4, must be returned by November 12, 1965.

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Room 301, Eland House,
Stag Place, London, S.W.1.

YOUNG GRADUATES REQUIRED with good knowledge of physico-chemical and analytic techniques for developing automatic measuring and control methods in fermentations and other biochemical systems. Excellent experimental facilities available, including comprehensive semi-industrial scale fermentation plant. Wide scope for the development of initiative and original ideas in association with an active research team. Salary according to age and experience.—Applications should be sent to the Secretary, Biochemistry Department, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7.

THE PUBLIC HEALTH LABORATORY Service Board require a Bacteriologist to work as Assistant to Dr. E. S. Anderson, Director of the Enteric Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W.9. Candidates should have at least three years' experience in bacteriology. Work mainly concerned with the phage-typing and epidemiology of salmonellae, with drug resistance and its transfer in the Enterobacteriacae, and with bacteriophage research. Good opportunities for original work. The post will be graded not below Senior Registrar (or equivalent for non-medical graduates) according to the age and experience of the applicant under terms and conditions similar to those of the N.H.S.—Further particulars may be obtained from the Secretary, P.H.L.S. Board, 24 Park Crescent, London, W.1, to whom applications should be sent, stating date of birth, qualifications, experience, and the names of three referees.

UNIVERSITY OF ALBERTA
DEPARTMENT OF CHEMISTRY

Applications are invited for the position of

PROFESSOR in Physical Chemistry

Candidates in the fields of Solid State, Structure of Liquids, Spectroscopy or Thermodynamics are favoured, but all applications will be considered. Starting salary according to qualifications, in the range $15,000 to in excess of $20,000. Facilities for research are excellent and generous support is obtained from Provincial and National sources. The Department has 30 members and is active in all the major divisions of chemistry.

Further particulars may be obtained from

Professor Harry E. Gunning, Head,
Department of Chemistry,
University of Alberta,
Edmonton, Alta., Canada.

GRACE

FOOD TECHNOLOGIST / MICROBIOLOGIST

W. R. GRACE are seeking a qualified male or female scientist with experience or interest in these fields. The person appointed will be based in the Flexible Packaging Laboratory at Park Royal, London. The work will involve problems and spoilage mechanisms found in food and packaging.

In this appointment, experience will be gained of other branches of scientific study as these refer to the newer developments in food packaging.

The company operates modern personnel policies. The fringe benefits include pension scheme, free life assurance, and free travel accident insurance.

Applications in strict confidence should be sent to

The Personnel Manager,
W. R. GRACE LIMITED,
ELVEDEN ROAD, PARK ROYAL, LONDON, N.W.10

UNIVERSITY OF CAMBRIDGE
ENGINEERING DEPARTMENT

The Department is receiving substantial grants from the Science Research Council for expanding its work on the application of computers to control and design in engineering. This work is directed at (1) the application of control theory to process optimisation, in association with plant operation and (2) the use of computers in engineering design using "light-pen" techniques.

An increase in staff is required to take full advantage of the new equipment being installed and there are several vacancies for research workers of proven ability. Some knowledge of control theory is desirable and this should be coupled with practical experience either in engineering design or in process operation.

There will be four senior appointments at the level of Assistant Director of Research, Senior Technical Officer and Design Engineer together with supporting staff. Appointments will be made within the salary ranges shown below.

Assistant Director of Research	**£1,740–£2,505**
Senior Technical Officer	**£1,740–£2,505**
Design Engineer	**£1,740–£2,505**
Senior Assistant in Research	**£1,400–£1,995**
Technical Officer	**£1,400–£1,995**
Assistant Technical Officer	**£950–£1,665**

Further details regarding these appointments and application form may be obtained from the Secretary of the Faculty Board of Engineering, University Engineering Department, Trumpington Street, Cambridge.

Allen Clark Research Centre

Materials Science

GRADUATE PHYSICIST

To investigate the phenomena occurring when relay contacts are closed and parted.
A suitable man must have some insight into plasma physics but should also be interested in the broad aspects of the elastic properties of relay structures and the metallurgy of contact materials.
The successful man will set up a small specialist department, and should in the future become the principal scientific adviser to the Company on all contact phenomena. He must have sufficient experience to run the job himself with appropriate assistance.
The laboratories are well equipped and are pleasantly situated in rural Northamptonshire within easy reach of the M1 and Northampton and several other towns. Company housing may be available.
For further details please write in confidence to: The Regional Personnel Manager, Allen Clark Research Centre, The Plessey Company Limited, Caswell, Towcester, Northants.

The Plessey Company Limited

ICI

AGRICULTURAL DIVISION

ICI

CHEMISTS FOR PESTICIDE RESEARCH

Chemists are required to lead teams engaged on pesticide research at Jealott's Hill Research Station, Bracknell, Berkshire. Over a period the work may include many aspects of surface chemistry associated with colloids, emulsions, dispersions and the complex problems involved in the application of pesticides to plants. Both fundamental and practical research is carried out and considerable freedom is given to the pursuit of personally developed projects. Close liaison with other specialists at the Research Station provides adequate opportunity for seeing all aspects of a research problem.

Applicants should have an honours degree level qualification in chemistry or pharmacy.

Please write, giving brief particulars only, to:
The Staff Officer, Plant Protection Limited, Fernhurst, Haslemere, Surrey.

ENTOMOLOGIST

BECHUANALAND

Required to undertake a survey of the cotton, maize and sorghum pests and make recommendations as to their control.

Candidates must hold a B.Sc. degree. Experience of African conditions is desirable.

Salary £1,680 to £2,260 a year, subject to British income tax, plus a tax free overseas allowance of either £235 (single), £600 (married unaccompanied) or £720 (married accompanied) a year. Passages provided. Education allowances. Two-year contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/19/06 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF EAST ANGLIA

SCHOOL OF MATHEMATICS AND PHYSICS

Applications are invited for posts of Reader, Senior Lecturer, Lecturer or Assistant Lecturer in Physics from October 1, 1966. The stipends will be on the scale: Reader, £2,550 by £100 to £3,250; Senior Lecturer, £2,450 by £100 to £3,150; Lecturer, £1,400 by £85 to £2,505; Assistant Lecturer, £1,050 by £75 to £1,275, plus F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C, with whom applications (one copy only), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 20, 1965.

THE BRITISH COKE RESEARCH ASSOCIATION invite applications for an appointment of a graduate inorganic or physical chemist or graduate physicist as a Scientific Officer in its Fundamental Studies Laboratory at Chesterfield. The duties are to collaborate in research in the general field of materials science and specifically in studies of oxidation of technical carbons or of their physical behaviour under changing stresses. The salary will be in accordance with the scientific Civil Service scales and will fall within the range £895 to £1,521 (Scientific Officer grade) £1,685 to £2,082 (Senior Scientific Officer grade) according to qualifications and experience. The post is superannuable.—Applications, giving age, education and experience, should be sent to the Secretary, The British Coke Research Association, Chesterfield, Derbyshire.

TOBACCO RESEARCH COUNCIL LABORATORIES

ORGANIC CHEMIST

The Tobacco Research Council invite applications from Research Chemists to fill a vacancy in the Chemistry Department of their Research Establishment at Harrogate.

Applicants should have a good honours degree in Chemistry with at least three years postgraduate research experience and preferably a Ph.D.

The successful candidate will join a team working on the isolation and characterization of constituents of tobacco smoke which may have a bearing on human health. Initiative, experience of modern chemical separation techniques and in particular Gas Chromatographic methods are required for this post.

The initial salary will be according to age, qualifications and experience and will be subject to annual review. Superannuation benefits will be made available under F.S.S.N.

Applications, giving full particulars of qualifications, age, experience and present salary should be addressed to:

The Chief Chemist,
Tobacco Research Council Laboratories,
Otley Road,
Harlow Hill,
Harrogate.

ASLIB

DEPUTY-HEAD OF RESEARCH DEPARTMENT

The Department is being substantially enlarged, and applications are invited for the new post of Deputy-Head. The successful candidate would have prospects of promotion on the retirement of the present Head of the Department in 1967.

The functions of the department are research, development, consulting and advisory work relevant to the collection, storage and dissemination of specialized information, largely but not exclusively scientific and technical, including investigations on the operations and techniques of special libraries and information services, and the introduction of mechanized systems. The department receives Government financial support. The Deputy-Head would have particular responsibility for planning, initiating and developing research programmes.

Suitable applicants are likely either to have held senior posts in library/information services and to have shown particular interest in library research; or to have had wide experience in the application of research techniques, particularly operational research, in other fields, and an interest in documentation.

The salary scale applicable to the post is: Deputy-Head, £2,335 to £3,192 per annum (Head: £3,385 to £3,885 per annum).

Intending applicants, before making formal application, should write for further particulars to the Director, Aslib, 3 Belgrave Square, London, S.W.1. Final applications should be received by November 15, 1965.

COMMONWEALTH AGRICULTURAL BUREAUX

Applications are invited for the following posts:

1. DIRECTOR, COMMONWEALTH BUREAU OF ANIMAL HEALTH, Weybridge, Surrey, England.

The work of the Bureau is to collect, collate and disseminate, through its publications and otherwise, information related to veterinary research, education and control of diseases of livestock. To carry out this work literature from all parts of the world has to be obtained and searched.

Applicants should be veterinarians (M.R.C.V.S. or equivalent qualification) and have knowledge of modern languages and ability to write concise English. Previous editorial and administrative experience are desirable.

2. DIRECTOR, COMMONWEALTH BUREAU OF SOILS, Harpenden, Hertfordshire, England.

The work of the Bureau is to collect, collate and disseminate, through its publications and otherwise, research information on soil and related sciences. To carry out this work literature from all parts of the world has to be obtained and searched.

Applicants must have a wide general knowledge of soil science. Experience of the principles and practice of classifying, storing and retrieving research information is most desirable, and a reading knowledge of several foreign languages would be regarded as an asset.

Salary £3,115 to £3,800 with provision for superannuation.

Applications should be submitted by December 31, 1965, to the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks, England, from whom full particulars, including regulations regarding provision of passages to oversea candidates on first appointment, can be obtained.

NEW ZEALAND

HEALTH DEPARTMENT

Applications are invited for the undermentioned vacancy:

Vacancy B 13/9/20/3908

FOOD TECHNOLOGIST, HEAD OFFICE WELLINGTON

Salary.—Up to £1,825 per annum.

Qualifications desired.—Graduate in food technology, or chemistry graduate with food industry experience. Duties include investigation of food processing in light of consumer protection legislation such as Food and Drugs Act; assessing justification for food additives; recommending minimum standards; liaison with industry and public agencies. Some travelling involved.

Passages.—Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses.—Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London S.W.1, with whom applications will close on November 22, 1965. Please quote reference B 13/9/20/3908 when enquiring.

BDH

BIOCHEMICALS

The active interest which the BDH Laboratory Chemicals Division has taken for a number of years in their production has greatly extended the availability of research biochemicals of high purity made in this country and will be continued still more actively in the future.

In this background the Division now needs the services of a well-qualified and experienced biochemist with a serious interest both in the field of biochemical research (and the applications, especially clinical and medical, of its results), and in the provision of British materials to meet the needs of biochemical laboratories throughout the world.

He will be required to advise on new developments and requirements; to prepare monographs on biochemical materials and subjects; to present papers on appropriate occasions; to maintain communications with research and other laboratories. He will have eventually to be prepared to travel, possibly frequently, both in this country and abroad.

It is expected that the most suitable candidates will be between 28 and 35. The salary paid will accord with qualifications and ability. Applications should be addressed to the Personnel Manager at Poole, and we would prefer to receive them before the 1st November.

THE BRITISH DRUG HOUSES LTD.,
BDH LABORATORY CHEMICALS DIVISION, POOLE, DORSET

FISONS PHARMACEUTICALS LIMITED

JUNIOR TECHNICIAN IN HISTOLOGY

FISONS PHARMACEUTICALS LIMITED, wish to appoint a JUNIOR TECHNICIAN in the Histology Section of their Research Laboratories.

Applications are invited from technicians possessing the intermediate examination of the I.M.L.T. (or equivalent) and desiring to specialize in Histology.

A wide range of histological and histochemical techniques are performed, also photomicrography and the preparation of tissue for electron microscopy.

Attractive salary scales are offered which compare favourably with the Whitley scales. In addition an annual Profit Sharing Bonus Scheme is in operation. The Company works a five-day week and the Fisons Group welfare schemes are applicable, including a living away allowance for employees under the age of 22. Three weeks annual holiday.

Applicants should apply to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF MECHANICAL ENGINEERING

Honours graduates in Engineering, Physics or allied subjects are invited to apply for two appointments as S.R.C. RESEARCH ASSISTANTS in the above department for the purpose of carrying out investigations under the direction of Dr. H. E. Rose, Professor of Powder Technology in the following fields of study (1) Electrostatic Precipitation, (2) Dispersion of Solid Particles in Fluids. Duties to commence as soon as possible.

Salary, depending on experience and qualifications, will be up to £1,000 per annum, and the appointments will initially be made for a period of two years.

Application forms may be obtained from the Registrar, King's College, Strand, W.C.2; completed forms must reach him by November 1, 1965.

TETLEY WALKER PRODUCTION LIMITED
WARRINGTON

GRADUATE ANALYTICAL CHEMIST

Young male or female Graduate Chemist required, to be responsible for chemical control work in small Brewery laboratory. A science degree with either postgraduate work or experience in Food or Chemical Industry will be necessary.

Excellent conditions of employment in new laboratories. Pension arrangements available. Salary in accordance with the scales of the British Association of Chemists.

Applications, giving full personal details, should be addressed to:

The Personnel Adviser,
Tetley Walker Ltd.,
The Brewery,
LEEDS 10.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF ANIMAL GENETICS

RESEARCH SCIENTIST

Applications are invited for appointment as Senior Research Scientist or Principal Research Scientist with the Organization's Division of Animal Genetics. The appointee will be located at the Division's Cattle Research Laboratories, Rockhampton, Queensland.

The Cattle Research Laboratory is engaged in studies of inherited characters of cattle affecting their general performance and adaptation to a tropical climate. The Laboratory is very well equipped and is air-conditioned. It operates in association with the National Cattle Breeding Station, "Belmont" Rockhampton, which carries about 1,400 head of well-controlled experimental cattle derived from Brahman, Africander, Hereford and Shorthorn breeds. The breeding programme is designed to provide material for studies of genetic differences. Good facilities for experimentation are available, and facilities for slaughter and associated work are being provided.

The appointee will be expected to develop research on the anatomy and patterns of growth of cattle with a view to clarifying genetic difference between individuals in carcase composition. Collaboration with other research staff working on related biochemical and nutritional problems is encouraged.

Applicants should have a Ph.D. degree or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of ability to initiate original research independently or in collaboration with other research workers. Experience should be in a relevant, but not necessarily closely related field.

SALARY : Dependent upon qualifications and experience within the ranges of :

£A3,235 to £A3,755 per annum or £A3,885 to £A4,485 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

Promotion within C.S.I.R.O. to a higher classification will be determined by merit.

Fares paid for the appointee and his dependent family. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.,

to whom applications (quoting Appointment No.: 682/28) should be addressed by December 18, 1965.

COMMONWEALTH OF AUSTRALIA

DEPARTMENT OF HEALTH

COMMONWEALTH ACOUSTIC LABORATORIES

PRINCIPAL PHYSIOLOGIST

Applications are invited for this position, located in the Physiology Sub-Section, Sydney.

Salary : £A3,296 to £A3,540.

Duties : Investigations into Acoustic Physiology, particularly the effects of noise and ultrasonics on people.

Qualifications : Ph.D., in Physiology with research experience, or Honours Degree with equivalent postgraduate research training and experience.

Fares: First class air/sea transport for the successful applicant and dependants (wife and dependent children) will be provided by the Commonwealth.

Applications : Forms can be obtained from and should be returned to : The Chief Medical Officer, Australia House, Strand, London, W.C.2, by November 12, 1965.

SOUTH AUSTRALIAN INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL TECHNOLOGY AND METALLURGY

Applications are invited for the following positions:

LECTURER IN METALLURGY
(ADELAIDE DIVISION)

Duties, to be responsible for teaching extractive metallurgy subjects in the course for B.App.Sc. in Primary Metallurgy, and to assist with lecturer and laboratory work in the Secondary Metallurgy degree course, and to other service courses provided by the Metallurgy Department. Qualifications, honours degree in metallurgy essential. Teaching, research or industrial experience an advantage, as would be an interest in the application of thermodynamics to extractive metallurgy processes.

Opportunities exist for research, and staff are able to study for higher degrees of the University of Adelaide.

LECTURER IN METALLURGY
(WHYALLA DIVISION)

Applicants should have experience in metallography ; some knowledge of chemical or mechanical metallurgy would be an advantage.

LECTURER IN METALLURGY
(WHYALLA DIVISION)

Applicants should have a primary interest in physical chemistry, and some experience of general analytical chemistry would be an advantage.

Applicants for appointment at Whyalla should possess a university degree and should be entitled to membership of an appropriate professional body.

Salary: £A2,400 by £A110 to £A3,170 per annum.

Leave: Annual leave, sick leave, and long service leave are allowed. After six years' service the appointee may apply for paid leave of absence for up to one year to obtain further experience or to pursue advanced studies. Financial assistance may be granted at the discretion of the Council.

Superannuation: The appointee will be eligible to join the South Australian Superannuation Fund.

Other Conditions: Assistance will be given with travelling and removal expenses.

Contract Employment: Consideration will be given to applicants desiring employment on a contract basis for a period of three years at a fixed salary of £A2,500 per annum.

Applications, giving personal, academic and professional details, together with the names and addresses of two referees, should reach the Registrar, South Australian Institute of Technology, North Terrace, Adelaide, South Australia by November 30, 1965. A second copy should be sent to the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

Further particulars may be obtained from the Registrar of the Institute in Adelaide or from the Association of Commonwealth Universities.

ROYAL VETERINARY COLLEGE

TECHNICIAN

required for Department of Medicine for duties associated with Animal Hospital. Salary scale £600 to £840 per annum. London Weighting (up to £45) and Qualification Allowance (up to £50) in addition. Superannuation scheme. Particulars of previous experience in writing as soon as possible to Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

UNIVERSITY OF TASMANIA

CHAIR OF ORGANIC CHEMISTRY

The University has established a Chair of Organic Chemistry within the Department of Chemistry and invites applications for appointment thereto. The research facilities of the department include spectrophotometers (Perkin-Elmer Models 4000A and 221G, Hilger Uvispek etc.), preparative and analytical vapour phase chromatography, etc. There is a well-equipped workshop. Professor H. Bloom, Ph.D., D.Sc., D.I.C., F.N.Z.I.C., F.R.A.C.I., Professor of Physical and Inorganic Chemistry, is the present head of the department. It is envisaged that the two professors will share administrative responsibilities by mutual agreement. The present professorial salary is £A5,200 per annum.

Information regarding duties and conditions of appointment in the University may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 22, 1965.

VICTORIAN COLLEGE OF PHARMACY

(SCHOOL OF THE PHARMACEUTICAL SOCIETY OF VICTORIA)

ASSOCIATE DEAN (PHARMACOLOGY)

£4,300 per annum.

Applications are invited for the position of Associate Dean (Pharmacology). The position involves the organisation, development, and leadership of the Department of Pharmaceutical Biology at the Victorian College of Pharmacy. This department is responsible for the lectures and practical work in Biology, Physiology and Pharmacology, which are taught in a three year full-time degree-type course. The Dean of the College has been acting as head of this department.

The successful applicant should have a higher degree (Ph.D. level) and would be expected to develop pharmacological research within the school.

The College is housed in a new building in pleasant surroundings at Parkville, Victoria, in close proximity to the University of Melbourne and the teaching hospitals attached to the University.

The College has a population of 500 students and is the only institute teaching pharmacy in Victoria.

The College's activities are organised into three departments, namely, Pharmaceutics, Pharmaceutical Chemistry, and Pharmaceutical Biology. Each department is responsible for one third of the student exposure time.

At present the Department of Pharmaceutical Biology has permanent but limited laboratories and research facilities. The school has recently been fully and adequately provided with the latest available equipment. It is proposed to build a new laboratory wing which would allow extensions. The successful applicant would be responsible for the planning and design of the appropriate portion of this wing. £3,000 is available immediately for the purchase of pharmacological research equipment.

Superannuation similar to F.S.S.U. is available.

The names of two referees are required.

The successful applicant would be expected to take up duties by February 1, 1966.

Applications and any further requests for information should be addressed to the Registrar, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria, Australia.

Closing date: November 30, 1965.

NEW ZEALAND

AGRICULTURE DEPARTMENT

Applications are invited for the undermentioned vacancy:

Vacancy B13/2/53/3798

SCIENTIST, RUAKURA AGRICULTURAL RESEARCH CENTRE

Salary: Up to £2,280 per annum, according to qualifications and experience. Further promotion to £3,100 per annum based on scientific merit.

Duties: To assist in a research programme in dairy cattle nutrition aimed at evaluating pasture as a feedstuff for growing, lactating and fattening stock and at measuring the voluntary intakes of grazing stock, the factors which affect these and the feed requirements of grazing animals. In addition to contributing in current projects the successful applicant will have the opportunity to develop independent lines of investigation.

Qualifications: Ph.D. or Honours degree preferably with some additional research experience in ruminant nutrition.

Passages: Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses: up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and General Information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London S.W.1, with whom applications will close on December 1, 1965. Please quote reference B13/2/53/3798 when enquiring.

HILL END HOSPITAL

ST. ALBANS, HERTS

Senior Biochemist holding appropriate science degree or other approved qualifications, required. The successful candidate will be based at Hill End Hospital which is within easy reach of Central London. Duties consist of routine and advanced investigations from other laboratories in the area. Research an advantage. Intending applicants may visit the laboratory by appointment. Salary £1,780 rising to £2,210 per annum.

Applications, stating age, qualifications, experience and naming two referees to Consultant Pathologist.

BRL

The discovery and development of a range of outstandingly successful antibiotics by Beecham Research Laboratories has made it one of the leading and fastest growing British ethical pharmaceutical houses. It markets these and its other products throughout the world.

SECTION HEAD MICROBIOLOGY

A vacancy will shortly arise for a man or woman to run the microbiological section of the Control Laboratory. Ten girls are employed in the section primarily on Assay and Sterility tests, although other services are provided for the production departments.

Candidates should hold a degree, or its equivalent, in a biological subject and have had at least two years' industrial, Government, or research experience since qualifying. Preference will be shown to those who have had experience of being responsible for the work of others. The Control Laboratory will be moving to new premises at Worthing, Sussex, in about a year's time and assistance will be given for this move.

Beecham Research Laboratories is a branch of Beecham Group for whose profit participation and non-contributory life assurance and pension schemes the successful candidate will qualify. An attractive starting salary will be offered. Application letters, giving details of experience and present salary, should be sent to the **Personnel Manager, Beecham Research Laboratories, Great West Road, Brentford, Middlesex.**

RESEARCH IN CHEMISTRY

There are vacancies for LABORATORY STAFF at the LONDON RESEARCH STATION of the GAS COUNCIL, which is situated at FULHAM.

Successful candidates will assist graduates in analytical research projects involving the use of thin layer chromatography, gas chromatography and similar techniques.

Experience in at least one of these techniques is essential.

Candidates should have obtained an "A" level in chemistry as a minimum qualification.

The starting salary will be according to age, qualification and experience, and there are excellent prospects for advancement.

Applications, giving full details should be sent to the Appointments Officer, North Thames Gas Board, 30 Kensington Church Street, W.8, quoting reference NE/2493.

GEOLOGIST

A large International Company in the Oil Industry with extensive interests throughout the world seek a GEOLOGIST with a Doctorate or good honours degree.

The selected candidate will join a team in the U.K. engaged on a project involving systematic stratigraphic analysis and tectonic interpretation. Experience of the Petroleum Industry would be an asset.

The position will involve some travel and may lead to an overseas posting.

Salary will be according to age, qualifications and experience, and the Company offers first-class terms of engagement and conditions of service.

Reply to Box No.: 10918, c/o 117 Piccadilly, W.1.

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

has vacancies of Scientific Officer grade for

(i) a RESEARCH MICROBIOLOGIST to join a team carrying out both basically-orientated and applied investigations in food microbiology. Applicants should have research experience, preferably in the field of microbial physiology.

(ii) a BIOCHEMIST to undertake an investigation into non-enzymatic oxidative reactions causing textural changes in preserved vegetable tissues. Applicants should have a higher degree or equivalent postgraduate experience.

The current salary scale for the grade is £844 to £1,395. The point of entry will depend upon age and qualifications. Both posts carry life assurance and contributory superannuation.

Applications should be addressed to the Director of Research, B.F.M.I.R.A., Randalls Road, Leatherhead, Surrey.

RESEARCH LEADER

MORGANITE RESEARCH AND DEVELOPMENT LIMITED

Battersea Church Road,
London, S.W.11

Morgan

ARE YOU A SENIOR SCIENTIST
with a flair for designing experiments which, whilst not necessarily complicated, are calculated to yield a maximum of relevant information? Perhaps you have just completed a Ph.D. or you may already have had some years of post-graduate research experience.

ARE YOU LOOKING
for a job that will give full range to your creative talents and, at the same time, bring you into the front line of industrial research and development?

IF SO
we have challenging work in the fields of rheology, powder technology and materials evaluation at temperatures up to and beyond 2000°C. Furthermore, we are confident that we can offer a salary attractive to suitably qualified men capable of leading research in these areas.

Applications, quoting reference N/QG5, should be addressed to the STAFF MANAGER.

PHYSICISTS
ELECTRICAL ENGINEERS

Posts are available on the Research Staff of the Association's Materials Department, for Graduates (or equivalent experience) interested in basic research into the following problems. Previous experience is not essential.

- Use of low-loss Plastics in the Megavolt/C M Range of stress. The work is directed toward the ultimate development of supertension equipment. Two vacancies exist, one concerned mainly with Physical and the other with Engineering aspects. The second might suit a Mechanical Engineer with interest in extrusion problems and some electrical knowledge.
- Study of the electro-chemical formation and electrical properties of very thin insulating films, especially of silicon oxide on silicon.

Appointments will be made either at Ph.D. or equivalent level at salaries up to £1,800 p.a. or at Honours Graduate level at salaries up to £1,300 p.a. depending on age and experience. Promotion to higher salary ranges is open to those who show leadership in original research.

Members of the Research Staff are encouraged to publish Scientific papers and to become recognised experts in their respective fields. The Laboratories are situated in very pleasant open surroundings in the London Green Belt near the North Downs. Some contribution towards costs of removal is available for married candidates.

If you are interested in any of the above Posts please write to or telephone

P. L. BYE, Personnel Officer,
ELECTRICAL RESEARCH ASSOCIATION,
Cleeve Road, Leatherhead, Surrey.
TEL: Leatherhead 4151

TIMBER MILLING INSTRUCTORS
CHILE

Two experts in saw milling, processing, seasoning and impregnation of wood are required to instruct students at the Timber Section of the Escuela de Concepcion, Chile. An appropriate degree or diploma and a knowledge of Spanish desirable but not essential. The assignment is for two years. Salary £1,500 per annum subject to U.K. income tax, plus a non-taxable foreign service allowance of £610 (single) £990 (married unaccompanied) and £1,180 (married accompanied). Passages provided. Accommodation available. Education allowances. Medical and dental treatment available.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting Ref. RC 324/41/01 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF ADELAIDE
(NORTH TERRACE AND BEDFORD PARK)
MATHEMATICS

Several vacancies exist at both institutions in permanent posts at various levels, in the fields of Pure Mathematics, Applied Mathematics, Statistics, Computation. While each institution has its own resources in terms of accommodation, secretarial help, libraries etc., and while they carry out teaching and research independently of each other they are co-operating closely in order to facilitate advanced teaching and research. At present there are about thirty postgraduate students. Research is in progress in the following fields (among others): Functional Analysis, Multivariate Statistics, Number Theory, Oceanography, Operations Research.

The salary scales currently in force are: Reader, £A4,300 per annum; Senior Lecturer, £A3,250 by £A110 to £A3,800 per annum; Lecturer, £A2,400 by £A110 to £A3,170 per annum. There is an attractive superannuation plan, a disablement pension plan and study leave on full pay with generous travel grants. Travel grants are paid to those coming to take up appointments.

Anyone interested in applying for a position should write in the first instance to either of the following:

Professor R. B. Potts, Department of Mathematics, The University of Adelaide, Adelaide, South Australia.

Professor B. Abrahamson, School of Physical Sciences, The University of Adelaide at Bedford Park, 199 North Terrace, Adelaide, South Australia.

UNIVERSITY OF ZAMBIA

Applications are invited for the following appointments: Senior Lectureship, Lectureship or Assistant Lectureship in Geography (two posts—preferably in Physical or Cartographic fields). Lectureship or Assistant Lectureship in Chemistry (two posts—one in Organic and the other in Physical or Inorganic Chemistry). Salary scales: Senior Lecturer, £2,350 by £100 to £2,750 per annum; Lecturer Grade I, £1,950 by £75 to £2,250 per annum, Grade II, £1,350 by £75 to £1,800 per annum; Assistant Lecturer, £1,100 by £75 to £1,250 per annum. A pensionable contract addition of £300 per annum is payable to non-Zambians who will be appointed on five-year contracts. Return family passages and allowance for transport of effects; installation grant of £100; part-furnished housing provided at rent of 7 per cent to 10 per cent of salary; home leave with passages provided every two years; provision for study leave; various allowances for children. Medical Aid Scheme. A Superannuation Scheme is being negotiated.

Detailed applications (six copies), naming three referees by November 12, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom full details may be obtained.

ROYAL MILITARY COLLEGE OF SCIENCE

ENGINEERING PHYSICS BRANCH
Professor C. Holt Smith, C.B.E.

Applications are invited for a post of Lecturer in Engineering Physics to lecture to students from the Services and Civilians pursuing postgraduate specialist courses and, after 1967, London University External Degree students taking Automatic Control. Research is encouraged and first-class facilities are available, including an Elliott 803 digital computer; the results can be published or submitted for a higher degree. Qualifications: first- or second-class honours degree in Engineering or an equivalent or higher qualification, or approved experience in the subjects covered by the Engineering Physics Branch, viz.: servomechanisms, analogue and digital computing (including simulators), visual and infra-red optical instrumentation and guided missile control. Experience of hydraulic and pneumatic control systems particularly desirable. Salary scale: £895 to £2,082. Starting pay according to age and experience, e.g., an applicant aged 27 with good qualifications and at least three years' postgraduate experience could start at £1,685 per annum. Prospects of promotion to the Principal Lecturer grade; salary range £2,174 to £3,002. Superannuation under F.S.S.U. Residential accommodation can be provided for a single man. Possibility of housing for a married man.

For further information write to the Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

UNIVERSITY COLLEGE CORK

SPECIAL TERMINABLE ASSISTANTSHIP IN APPLIED PSYCHOLOGY

The Governing Body invites applications for the above full-time post with duties in either Industrial or Educational Psychology. Salary £1,200 by £125 to £1,450 per annum.

Before submitting applications candidates should first obtain from the undersigned particulars as to the requirements for the filling of the vacancy. Latest date for receipt of completed applications is 12 noon on Friday, November 12, 1965.

D. L. WHITE,
Secretary.

UNIVERSITY OF SHEFFIELD
DEPARTMENT OF PHYSICS
EXPERIMENTAL OFFICERS

Applications are invited for two posts of Experimental Officer, to be responsible for the operation of laboratories in the Department of Physics. Applicants would normally possess a University degree of its equivalent, although in exceptional circumstances particularly wide practical experience of the techniques involved could be accepted as an alternative. The two posts relate to (a) the use and general application of x-ray diffraction techniques and (b) the development of methods of growing crystals and the preparation of special materials. In each case the work is concerned with a wide range of problems which arise from the Department's research programme in Solid State Physics. Initial salaries according to qualifications and experience on the scale £870 by £55 to £1,200 by £75 to £1,275 bar by £60 to £1,695 bar by £85 to £1,950, with F.S.S.U. provision.

Further particulars may be obtained from the Registrar, to whom applications (four copies), should be sent by November 15, 1965.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF ENTOMOLOGY

Appointments in the fields of:

1. ECOLOGY AND BIOLOGICAL CONTROL

2. PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited for appointment to several research positions with the Organization's Division of Entomology located at Canberra, A.C.T.

The Division of Entomology is the main centre in Australia for basic research on insects and is also actively engaged on many problems of economic importance; the professional staff numbers about sixty. The Division's research programme ranges over the field of entomological research with particular emphasis at the present time in the fields of population dynamics and ecology, physiology and biochemistry, taxonomy, and biological control. Many studies are in progress on methods of insect control that do not rely upon the use of insecticides. There are excellent facilities for a wide range of research activities.

The choice of a specific problem within the broad fields of physiology and biochemistry, ecology, and biological control of insects, will be influenced by the interests and background of the appointee. In this connection applicants could, with advantage, have interests in one or other of the following: feeding behaviour of plant-eating insects; plant resistance factors; sex attractants or other pheromones; physical methods of control; genetics (meiotic drive in relation to insect control), and insect pathology.

Applicants should have a Ph.D. degree, or have had postgraduate experience of equivalent standard and duration supported by satisfactory evidence of research ability.

Salary: Dependent upon qualifications and experience within the salary ranges of:

£A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum

Salary rates for women are £A201 per annum less than the corresponding rates for men.

Promotion within C.S.I.R.O. to a higher classification is determined by merit.

The duration of the appointment will be determined in consultation with the successful candidate; some applicants may prefer a fixed term appointment for three years, in which case consideration will be given to the continuation of F.S.S.U. arrangements.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of a fixed-term appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.
to whom applications (quoting Appointment No.: 180/328, should be addressed by November 20, 1965.

TECHNICIAN—PARASITOLOGY

FISONS PHARMACEUTICALS LIMITED, manufacturers of ethical pharmaceuticals, have a vacancy in their Research Laboratories at Holmes Chapel, Cheshire, for a Laboratory Technician in the Parasitology Section.

The candidate (male or female) should have passed a final in Bacteriology or Parasitology or be completing studies for A.I.M.L.T., or equivalent qualification.

Practical experience in one or more human or veterinary parasitic infections would be an advantage but is not essential.

Applications should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, nr. Crewe, Cheshire.

UNIVERSITY OF NEW ENGLAND

Armidale, New South Wales

Applications are invited for the following positions:

PROFESSOR OF BIOCHEMISTRY: The person appointed will occupy the Foundation Chair in Biochemistry which is taught in both the Faculties of Science and Rural Science.

ASSOCIATE PROFESSOR OR SENIOR LECTURER OR LECTURER IN GEOPHYSICS: Preference will be given to applicants possessing a strong background in Geology and Physics, and with experience in exploration geophysics and/or the geophysics of the earth's crust and mantle. It is envisaged that Geophysics will develop as a separate Department within the Faculty of Science. The appointee initially will provide relevant parts of undergraduate courses in Geology at the second- and third-year level, and will participate in the development of Geophysics at the honours and postgraduate level.

LECTURER OR SENIOR LECTURER IN ZOOLOGY (TWO POSITIONS): Some special interest and experience in either Invertebrate Zoology, with special emphasis on Comparative Physiology, or Vertebrate Zoology, with special emphasis on Embryology, would be an advantage. The successful applicants will be required to take part in undergraduate teaching at all levels and to participate in the training of postgraduate students while developing their own research interests.

LECTURER/SENIOR LECTURER IN AGRONOMY: Applicants should have considerable research experience in the field of pasture and animal relationships. The successful candidate would be expected to organize and be responsible for a course of lectures in Pasture Agronomy, to develop research work in this field and to supervise postgraduate students studying for higher degrees.

LECTURER IN GEOLOGY: The appointment is to be made in the field of Sedimentary Petrology, Sedimentation and/or Lithostratigraphy. The appointee will give undergraduate courses and supervise postgraduate students to the Ph.D. level in the above fields, and undertake such other duties as may be assigned by the Head of the Department.

LECTURER IN PHYSIOLOGY: Applicants should have a pass or Honours degree in Physiology, Medicine, Veterinary Science, Rural Science or Agricultural Science and preferably with some experience in teaching or research. The Department of Physiology teaches in the faculties of Science and Rural Science and provides both a pass and an Honours course. Facilities are available for study towards M.Sc. and Ph.D. degrees, especially in the fields of environmental, reproductive blood and digestive physiology and the physiology of adaptation. The successful applicant will be encouraged to teach and work in one of the existing fields of interest within the Department, or to develop teaching and research in his own field.

TEACHING FELLOW OR DEMONSTRATOR IN PHYSIOLOGY: Applicants should have a pass or Honours degree in Physiology or a related discipline, Medicine, Veterinary Science, Rural Science or Agricultural Science. The Department of Physiology teaches in the faculties of Science and Rural Science and provides both a pass and Honours course. Facilities are available for studying for M.Sc. and Ph.D. degrees especially in the fields of environmental, reproductive, blood and digestive physiology and the physiology of adaptation.

TEACHING FELLOW OR DEMONSTRATOR IN MICROBIOLOGY OR PARASITOLOGY AND PATHOLOGY: The appointee will, depending on qualifications, be required to demonstrate in Microbiology or Parasitology and Pathology and possibly in Anatomy.

GENERAL CONDITIONS: Salary: Professor, £A5,200; Associate Professor, £A4,300; Senior Lecturer, £A3,300 by £A100 to £A3,800; Lecturer, £A2,400 by £A110 to £A3,170; Teaching Fellow/Demonstrator, £A1,500 by £A100 to £A1,900.

Commencing salary will be determined according to qualifications and experience. First class fares will be paid and a contribution will be made towards removal expenses (up to £A200 for appointees from within Australia and up to £A300 for appointees from overseas). Provision is made for superannuation, for assistance in buying or building a home and for study leave. Study leave travel grants are available and credit may be given for existing study leave entitlement.

Further information may be obtained from the Registrar of the University, with whom applications (two copies, the names of three referees and a passport size photograph) close on November 26, 1965. Applicants in the United Kingdom should also forward an additional copy of the application to the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. (from whom further particulars may also be obtained) by the same date.

DEPARTMENT OF PHYSICS

UNIVERSITY OF WINDSOR
WINDSOR, ONTARIO, CANADA
GRADUATE ASSISTANTSHIPS

The Department of Physics offers Teaching and Research Assistantships to students qualified for admission to graduate studies and wishing to proceed to the M.Sc. or the Ph.D. in Physics. Research is proceeding in atomic collisions and atomic physics, nuclear spectroscopy, electron spin resonance, mass spectroscopy, plasma diagnostics and in several branches of theoretical physics. Stipends up to $3,500 per annum, depending on qualifications and duties in the department.

Inquiries should be addressed to Professor L. Krause, Head, Department of Physics.

UNIVERSITY OF LONDON

CHAIR OF BIOLOGY AS APPLIED TO MEDICINE AT MIDDLESEX HOSPITAL MEDICAL SCHOOL

The Senate invite applications for the Chair of Biology as applied to Medicine tenable at the Middlesex Hospital Medical School (salary not less than £3,400 a year plus £100 London Allowance).

Applications (ten copies), must be received not later than November 17, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF HULL

DEPARTMENT OF PHYSICS

Senior Experimental Officer or Experimental Officer required for electron microscope laboratory providing a service for physics, chemistry and biological departments. Applicants should have had experience of the use of an electron microscope in one of these fields and should preferably have a degree or equivalent qualification. Salary scale: Experimental Officer, £890 by £55 to £1,275; Senior Experimental Officer, £1,250 by £70 to £1,950.

Applications, together with the names of two referees, should be made to the Registrar, from whom further particulars may be obtained, by November 13, 1965.

AGRICULTURAL RESEARCH COUNCIL

Assistant Experimental Officer required to assist with chemical analyses in connection with biochemical studies on meat. The post is tenable in Cambridge, transferring in about two years' time to a new Meat Research Institute to be built at Langford near Bristol. Qualifications: If aged 22 or over, Pass degree or H.N.C. in chemistry, candidates under 22 two "A" levels in scientific subjects. Salary according to age £549 at age 18; £983 at 26 or over; maximum £1,201. Voluntary contributory superannuation scheme.

Application forms from Secretary, Low Temperature Research Station, Downing Street, Cambridge, quoting M65/10.

INSTRUCTOR, FORESTRY TRAINING SCHOOL

SIERRA LEONE

Applications are invited from suitably qualified persons to run the School for Forest Rangers. Candidates should hold a degree in Forestry but non-graduate Foresters with teaching experience will be considered. Salary £1,500 to £2,000 according to qualification subject to U.K. income tax, plus a variable non-taxable foreign service allowance of £695 single, £1,205 to £1,255 if married unaccompanied and £1,370 to £1,380 if married accompanied. Passages provided. Quarters available. Education allowances. Two to three year contract.

Candidates, who should normally be nationals of, and permanently resident in the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting Ref.: RC 324/159/02 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

MIDDLESEX HOSPITAL MEDICAL SCHOOL

LONDON, W.1

THE INSTITUTE OF NUCLEAR MEDICINE is searching for a young scientist—preferably a biochemist but a physicist with chemical leanings would be welcome—to assist in the development of micro-assay methods for hormones and vitamins using radioactive, immunological, chemical and computer techniques. The selected graduate will work in a small, enthusiastic and demanding team in which lack of interest but not of experience would be a disadvantage. Junior Lecturer scale though higher for the right person if older.

Applications with two references to the Secretary, Institute of Nuclear Medicine.

UNIVERSITY OF LEEDS

CENTRAL UNIVERSITY TELEVISION SERVICE

Applications are invited for the post of Assistant Producer. The successful applicant will learn television production techniques and, after initial experience under professional supervision, should be capable of taking charge of the Service's mobile camera unit. Candidates must be graduates, preferably in one of the fields of science or technology, and should have some experience of audio-visual systems and a keen interest in visual communication. Salary on the scale £1,050 by £75 to £1,275. Initial appointment at any point on the scale.

Applications (three copies), stating age, qualifications and experience, and naming three referees, should reach the Registrar, The University, Leeds 2 (from whom further particulars may be obtained), not later than November 15, 1965.

AGRICULTURAL ADVISER

TONGA

Required to advise and assist the Government of Tonga on agricultural development, including the organization and administration of the Department of Agriculture, implementation of agricultural development programmes, development of extension services and marketing of produce.

Candidates should have a degree in Agriculture and considerable agricultural and administrative experience both in the field and in the running of a department of Agriculture. Good experience with coconuts is desirable.

Salary £2,150 a year, subject to British Income Tax, plus a tax free foreign service allowance ranging from £240 to £735 a year according to marital status. Appointment for two years. Passages provided. Education allowances. Quarters provided.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving brief particulars of qualifications and experience, quoting RC 213/177/02 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

VICTORIA UNIVERSITY OF WELLINGTON
NEW ZEALAND
LECTURER IN ZOOLOGY

Applications are invited for the above-mentioned post. Preference will be given to candidates with special interests and qualifications in terrestrial zoology with emphasis on entomology, especially the ecology of insects. Opportunity will be given for the appointee to undertake research. The salary scale for a Lecturer is £1,300 rising to £2,000 per annum, with a bar, then on recommendation to £2,200 per annum, initial salary being determined according to qualifications and experience. In the case of a person appointed from outside New Zealand salary will be paid from the date of embarkation. Approved fares to Wellington will be allowed for the appointee and his family, together with actual removal expenses within specified limits.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in New Zealand and London on November 30, 1965.

UNIVERSITY OF NEWCASTLE UPON TYNE
SCHOOL OF PHYSICS

Applications are invited for the post of Lecturer in Geophysics in the School of Physics. It is expected that the successful applicant will possess high academic qualifications and will take an active part in the undergraduate and postgraduate teaching programmes, and also in the field of geophysical research. The commencing salary will be at an appropriate point on the scale £1,400 to £2,505 according to age, qualifications and experience. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne 2, with whom applications (three copies), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 9, 1965.

ENTOMOLOGIST

The International Office of Cocoa and Chocolate (the international association of the chocolate manufacturing industry) is sponsoring a team of scientists to carry out further research into the capsid pests of cocoa in West Africa, and wishes to engage an experienced entomologist with an interest in ecology for work in Nigeria. The appointment will be for a period of about three years, and the terms and conditions of service will be similar to those laid down by the Ministry of Overseas Development.

Applications, stating age, qualifications and experience, should be sent to the Director-General, International Office of Cocoa and Chocolate, 11 Green Street, London, W.1.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE
DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS

Applications are invited for Lecturer or Senior Lecturer in the Department of Pure Mathematics and Mathematical Statistics. Applicants should have qualifications and research interests preferably in some branch of mathematical statistics, but candidates with interests in applied statistics will also receive consideration.

Applications (six copies) containing the names of three referees, should be received not later than November 13, 1965, by the Registrar, University College, Cardiff, from whom further particulars may be obtained.

STRACATHRO HOSPITAL
BRECHIN, ANGUS (526 Beds)

Applications are invited for the post of Senior Biochemist attached to the Biochemical Department in the above hospital and with duties in the corresponding Department in Maryfield Hospital, Dundee. Salary and Conditions of Service according to current Whitley Council agreements.

Applications, giving age, qualifications and experience, together with the names and addresses of two referees, should be submitted to the Group Medical Superintendent, Stracathro Hospital, Brechin, Angus, from whom further particulars can be obtained.

ABBOTT LABORATORIES LIMITED
have a vacancy for a
DEPUTY MANAGER—QUALITY CONTROL

Applicants, aged 27 or over, should have, as a minimum, a University honours degree in Chemistry or an equivalent qualification and have spent several years working in a Quality Control Department, preferably in the Pharmaceutical Industry.
After a period of 1–2 years, promotion to Quality Control Manager is expected.
Commencing salary will be agreed by negotiation. The company has an attractive Pension Scheme with free life insurance and other benefits. Applications, stating age and giving details of qualifications and previous experience, should be addressed to :

The Personnel Manager,
ABBOTT LABORATORIES LIMITED,
Queenborough, Kent, Marking the application N7.

UNIVERSITY OF NEW SOUTH WALES
LECTURER IN THE DEPARTMENT OF ZOOLOGY
SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited for appointment to the above-mentioned position. Salary: £A2,400 range £A3,170 per annum. Commencing salary will be according to qualifications and experience. Applicants must have a distinguished academic record. Preference will be given to applicants with a research interest in one of the following fields: vertebrate physiology, embryology or tissue culture; cell biology; invertebrate zoology (other than entomology or marine biology). The successful applicant will teach undergraduate courses to honours level and supervise postgraduate students; he will also have the opportunity to do research in his own chosen field. The newly appointed Professor of Zoology is Dr. G. B. Sharman and facilities for research on the biology of marsupials are being established.

Details of appointment, including superannuation, study leave, housing scheme may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 30, 1965.

UNIVERSITY OF THE WITWATERSRAND
JOHANNESBURG
DEPARTMENT OF CHEMISTRY INCLUDING BIOCHEMISTRY
VACANCY: LECTURER IN BIOCHEMISTRY

Applications are invited for appointment to the post of Lecturer in Biochemistry in the Department of Chemistry including Biochemistry. Duties are to be assumed on January 1, 1966 or as soon as possible thereafter. The salary scale attached to the post is R.2,700 by R.150 to R.4,050 (R.2 =£1 stg.). The initial salary will be determined in accordance with the qualifications and experience of the successful candidate. In addition an annual vacation savings bonus is payable in terms of Government regulations.

Applicants are advised to obtain a copy of the information sheet relating to this post from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in South Africa and London on November 12, 1965.

UNIVERSITY OF HULL
TECHNICAL STAFF—
EDUCATION OFFICER

Applications are invited for a new post as Technical Staff Education Officer in the Faculty of Science, from a date to be arranged. Candidates should have a University degree in one of the physical or biological sciences, broad experience of science teaching, and good experimental technique. Salary scale: £1,250 by £70 to £1,950 per annum with F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, The University, Hull, to whom applications (three copies) should be made by October 31, 1965.

UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF PURE MATHEMATICS

Applications are invited for the Chair of Pure Mathematics which has become vacant through the resignation of Professor Bonsall on his appointment to the M'Laurin Chair at Edinburgh University. The person appointed will be required to take up duty as soon as possible and not later than August 1, 1966. Salary in accordance with professorial scale (£3,400 by £125 to £4,025 per annum) with membership of F.S.S.U.

Further particulars may be obtained from the undersigned with whom applications (15 copies), giving the names of not more than three persons to whom reference may be made, must be lodged not later than Saturday, November 20, 1965. (Applicants from outside the British Isles may submit one copy only.)

E. M. BETTENSON,
Registrar.

University of Newcastle upon Tyne,
6 Kensington Terrace,
Newcastle upon Tyne 2.

LONDON BOROUGH OF BARKING
SOUTH-EAST ESSEX COLLEGE OF TECHNOLOGY
Longbridge Road, Dagenham

Required as soon as possible, Lecturer in Biochemistry to teach the subject to the standard required for H.N.C. and H.N.D. in Applied Biology and to assist in the development of Biochemistry as part of the proposed M.I.Biol. and Honours Degree courses. Research interests desirable. Salary grade (at present under review): £1,670 by £45 to £1,895, plus London Allowance.

Further particulars and application form from the Principal at the College on receipt of a stamped addressed envelope.

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS

Scientific Officer with good honours degree in Botany, Agricultural Botany or Zoology to work on virus diseases of plants especially grasses. Starting salary according to experience on scale £895 to £1,521. Superannuation. F.S.S.U.

Applications, quoting reference 1051/62, with names and addresses of two referees, to the Secretary, by November 30.

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING

Applications are invited for the post of Lecturer or Assistant Lecturer in Electrical Engineering in the above Department. Salary £1,400 to £2,505 (Lecturer) or £1,050 to £1,275 (Assistant Lecturer) plus membership of the F.S.S.U.

Conditions of appointment, and form of application, to be returned by November 12, 1965, from the Registrar.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

(Proposed Welsh University of Technology)

ELECTRONIC SECTION

DEPARTMENT OF APPLIED PHYSICS

Applications are invited for a Research Demonstratorship in Electronics. Applicants must have a good degree (or equivalent) and have had not less than one year's experience in Research. The Demonstrator will be required to undertake research and laboratory demonstration and tutorial work. The appointment will be made for a specific period (normally not more than three years) at a salary of £700 by £50 to £800 per annum.

Application forms, to be returned by November 5, 1965, and conditions of appointment, may be obtained from the Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

UNIVERSITY OF ZAMBIA

Applications are invited for the Chairs of (i) Agriculture and (ii) Botany. Inquiries welcomed from persons interested in secondment for a limited period. Appointments to be assumed as soon as possible. Salary £3,300 per annum. A pensionable contract addition of £300 per annum is payable to non-Zambians. Return family passages and allowance for transport of effects; installation grant of £100; part-furnished housing provided at rent of £300 per annum; home leave with passages provided every two years; provision for study leave; various allowances for children. Medical Aid Scheme. A Superannuation Scheme is being negotiated.

Detailed applications (eight copies), naming three referees by November 12, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom full details may be obtained.

CITY OF SHEFFIELD

WATERWORKS UNDERTAKING

APPOINTMENT OF CHIEF CHEMIST AND BACTERIOLOGIST

Applications are invited for this appointment, scale "D" £1,940 to £2,280 on the permanent staff of the General Manager and Engineer (A. B. Baldwin, M.Eng.). Applicants must possess a science degree (Hon. Chem.) or be Members of the Institute of Chemistry and have had considerable practical experience in the operation of a laboratory, in the physical, chemical and bacteriological examination of water samples and of water treatment and purification processes. The appointment is superannuable and subject to medical examination and to the National Joint Council Conditions as applied by the City Council. A car allowance is payable.

Applications, stating age, education and training, professional qualifications, experience, present and past appointments (with dates and salaries), and quoting the names of two referees, should reach the undersigned by October 31, 1965.

SYDNEY HILTON,
Town Clerk.

Town Hall,
Sheffield 1.

BALDERTON HOSPITAL

NR. NEWARK, NOTTS

BIOCHEMIST—RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to take part in a programme of research at Balderton Hospital, designed to study human chromosome abnormalities and associated disorders in mental health. Experience in cytology desirable but not essential. This project is operating in close association with the Department of Genetics, Sheffield University. Initial salary for entrants with first- or second-class honours degree or, for Biochemist only, Associate or Graduate of the Royal Institute of Chemistry £935 per annum (£830 for other entrants). Additional increments for completed years of postgraduate study.

Further details can be obtained from Dr. H. Hunter to whom applications should be addressed as soon as possible.

APPLICATIONS ARE INVITED FOR Appointments combining teaching and research in the Department of Anatomy of an expanding Health Sciences Centre. The department is responsible for instruction in gross anatomy, histology, oral histology, embryology, neuroanatomy and certain more specialized courses to students in several Faculties. Research encouraged. Rank and salary commensurate with training and experience.—Write, giving pertinent information, to Dr. M. L. Barr, Head, Department of Anatomy, Health Sciences Centre, University of Western Ontario, London, Canada.

MATHEMATICIAN

A first-class applied mathematician is required by the Mining Research Establishment, National Coal Board, Worton Hall, Worton Road, Isleworth, Middlesex, to study problems arising from increased mechanization and concentration of mining processes. The work is mainly concerned with boundary value problems some of which involve the use of numerical methods; it covers a wide field and to date studies have been carried out in elasticity, stress waves, the properties of granular materials, gas diffusion and heat conduction. Encouragement is given to members of the staff to publish the results of their work. Starting salary according to ability and experience between £1,485 to £2,865; conditions of service are attractive. Applicants should preferably have had postgraduate training in research.

Applications, giving full details of qualifications and experience should be sent to the Headquarters Staff Manager (X.356F), National Coal Board, Hobart House, Grosvenor Place, London, S.W.1.

RESEARCH OFFICERS—GRADE II

TANZANIA

(TANGANYIKA AND ZANZIBAR)

Required to work at one of the four Livestock Breeding Stations on general farm management development and multiplication of improved breeds of cattle, maintenance of records and statistics and minor research projects into animal reproduction, genetics, nutrition and physiology.

Candidates must hold a degree in Agriculture with preferably some postgraduate experience in research work into livestock reproduction, nutrition, physiology and genetics.

Salary scale, £1,329 to £2,757 a year plus 25 per cent terminal gratuity. Passages provided. Education allowances. 21 to 27 months contract. Generous leave.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/173/01 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

ST. BARTHOLOMEW'S HOSPITAL

LONDON, E.C.1

TECHNICIAN for new laboratory associated with Department of Anaesthesia for research into respiratory and blood/gas abnormalities. Experience in blood/gas measurements and/or possession of Final I.S.T. or equivalent preferred. Salary scale in the range £415 to £1,000 per annum according to experience and qualifications.

Write, naming two referees, to the Clerk to the Governors, quoting Project No. 843.

BRITISH LEATHER MANUFACTURERS' RESEARCH ASSOCIATION

Graduate in Chemistry or Biochemistry, or Dip.Tech. in Applied Chemistry required for research on composition of animal skins and chemical modifications of collagen. Work suitable for higher degree. Salary in range £950 by £75 to £1,850.

Applications to Director, BLMRA, Milton Park, Egham, Surrey.

ROYAL COLLEGE OF ADVANCED TECHNOLOGY

SALFORD

(Proposed University of Salford)

DEPARTMENT OF ELECTRICAL ENGINEERING

Applications are invited from persons with suitable experience and qualifications for academic posts in the Electrical Engineering Department. Candidates should have a good degree in electrical engineering or physics, though for certain posts a mathematics degree would be appropriate. Research experience is normally required, but candidates who have recently graduated are eligible for consideration as Assistant Lecturers. The persons appointed will be expected to undertake original work and to teach undergraduate and postgraduate classes. The lecturing timetable, and laboratory facilities, are such that a genuine opportunity exists for the carrying out of original work. Candidates may be specialists in any field, though applications from specialists in Control Systems and in Power Systems are specially welcome. Appointments may be made within one of the following scales: Assistant Lecturer, £1,050 to £1,275 per annum; Lecturer, £1,400 to £2,505 per annum; Senior Lecturer, £2,450 to £3,250 per annum; Reader, £2,450 to £3,250 per annum.

Inquiries, which will be treated in confidence, should be made to Professor J. H. Calderwood at the College.

PROPOSED UNIVERSITY OF BRADFORD

(BRADFORD INSTITUTE OF TECHNOLOGY)

A Technician/Senior Technician is required in the Department of Biological Sciences. Duties involve assistance with Teaching Laboratories and relating classes, researches in biochemistry, controlled environment work and instrumentation and with the development of bioassay and culture techniques. Further information obtainable from the Professor of Biological Sciences. Salary according to qualifications and experience in range: Senior Technician, £840 to £1,040; Technician, £600 to £840, with an increment of £30 or £50 in either case for approved qualifications.

Application forms from the Secretary, Bradford Institute of Technology, Bradford, 7, to whom they should be submitted as soon as possible.

MANCHESTER UNIVERSITY

FACULTY OF TECHNOLOGY

DEMONSTRATOR IN CERAMICS

A vacancy exists for a temporary demonstrator to assist in laboratory work for a postgraduate course in Ceramics. A first degree in Metallurgy or Physics with laboratory experience in one or more of the following fields: X-ray Analysis, Electron Microscopy or Powder Metallurgy, would be as acceptable as previous experience in Ceramics. A substantial part of the time may be devoted to working for a higher degree. The post is tenable for twelve months in the first instance and renewable subject to satisfactory progress. Salary £850 per annum. Subject to superannuation under the Federated Superannuation System for Universities.

Applications should be sent immediately to the Registrar, Manchester College of Science and Technology, Sackville Street, Manchester, 1.

UNIVERSITY OF NEW SOUTH WALES

SENIOR LECTURER/LECTURER

SCHOOL OF TEXTILE TECHNOLOGY

Applications are invited for appointment to the above-mentioned position in the field of textile physics. Salary: Lecturer, £A2,400 range £A3,170 per annum. Senior Lecturer, £A3,300 range £A3,800 per annum. Commencing salary will be according to qualifications and experience. Applicants must have a distinguished record in physics or textile physics.

Details of appointment, including superannuation, study leave, housing scheme may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on December 12, 1965.

RESEARCH WORKER REQUIRED, PREferably with an interest in histology or neurology and with some photographic experience to work upon problems concerned with the localization of radioactive materials in tissues. Candidates should have a biological or medical background and the project is suitable for a higher degree.—Application forms from the Secretary, The Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5. (Ref. Psy.)

HEINZ 57 SCIENTISTS FOR RESEARCH AND DEVELOPMENT

Heinz need scientific staff for research into food processing and packaging at their new research centre recently opened in pleasant parkland at Hayes, Middlesex. Laboratory and pilot shop facilities are excellent. However, research and investigations cannot be carried out with modern equipment alone. They need more well-qualified scientific staff, specifically:

BIOCHEMISTS · CHEMICAL ENGINEERS
CHEMISTS · FOOD SCIENTISTS
PHYSICISTS · PHYSICAL CHEMISTS
MICROBIOLOGISTS

Work covers most technological aspects of food processing and, in particular, the Physicist/Physical Chemist will be engaged on investigations into technological aspects of food packaging. Applicants should have at least a good honours degree or appropriate Diploma of Technology. Research experience or Ph. D. would be an asset for the Biochemical post. The upper age limit in all categories is 32, although this is by no means inflexible. Salaries and working conditions are those of a large progressive company. Financial assistance in changing location can be granted in certain cases.

Apply in writing, giving a brief personal history, to:
The Personnel Officer,
H. J. HEINZ COMPANY LTD.
Hayes Park, Hayes, Middlesex

UNIVERSITY OF HULL
DEPARTMENT OF PHYSICS

Senior Experimental Officer or Experimental Officer required for supervision of crystal-growing laboratory. Applicants should preferably have a degree in physics or chemistry, an equivalent qualification, or have had relevant experience in the field of single crystal, powder phosphor and photoconducting material preparation.

Salary scales: Experimental Officer, £890 by £55 to £1,275; Senior Experimental Officer, £1,250 by £70 to £1,950.

Applications, together with the names of two referees should be made to the Registrar, from whom further particulars may be obtained by November 13, 1965.

UNIVERSITY OF EAST ANGLIA
SCHOOL OF MATHEMATICS AND PHYSICS

Applications are invited for a number of posts as Lecturer or Assistant Lecturer in Mathematics in the School of Mathematics and Physics from October 1, 1966. For one of the posts some preference may be given to candidates with research interests in algebra. The stipends will be on the scale: Lecturer, £1,400 by £85 to £2,505; Assistant Lecturer, £1,050 by £75 to £1,275, plus F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C, with whom applications (one copy only), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 20, 1965.

PLYMOUTH GENERAL HOSPITAL. Radiotherapy Department. Physics Technician (basic or senior grade) required to assist in some of the routine work with electronic and isotope equipment, and possibly additional technical duties in radiotherapy. Qualifications required: O.N.C. in electrical engineering or applied physics or equivalent. Salary scale £668 to £1,061 (at present under review) according to qualifications and experience.—Further details may be obtained from the Principal Physicist, Radiotherapy Department, Plymouth General Hospital, Plymouth.

BOTANISTS/PLANT BREEDERS

TANZANIA

(TANGANYIKA AND ZANZIBAR)

Required (1) to work on the improvement of general crops,

(2) to take charge of the maize breeding programme with a view to producing synthetic or hybrid varieties.

(3) to start a cashew breeding programme.

Candidates must hold a degree in Natural Science or Agricultural Botany, with at least two years' postgraduate plant breeding and selection experience.

Salary £1,329 to £2,757 a year plus a 25 per cent terminal gratuity. Passages provided. Education allowances. Generous leave 21 to 27 month contract. Government quarters.

Applicants, who should be nationals of the U.K. or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/173/05 to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY COLLEGE HOSPITAL
Gower Street, London W.C.1
OBSTETRIC HOSPITAL

Laboratory technician required for routine steroid hormone estimations.

Applications, with names of two referees, to Assistant Secretary.

PORTSMOUTH COLLEGE OF TECHNOLOGY
DEPARTMENT OF CHEMISTRY

Applications are invited for the following two posts; duties to commence as soon as possible:

SENIOR LECTURER/ LECTURER IN INORGANIC CHEMISTRY

Successful candidates will be expected to undertake research and to teach inorganic chemistry up to Honours degree level. A research interest in transition metal chemistry is desirable for one of these posts. The department is moving shortly into new well-equipped laboratories.

Salary scale: Senior Lecturer, £2,140 to £2,380 per annum; Lecturer, £1,875 to £2,140 per annum.

Application forms and further particulars from the Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth.

DUNDEE ROYAL INFIRMARY
CLINICAL CHEMISTRY

Applications are invited for the post of Principal Biochemist, Dundee Royal Infirmary. Whitley salary and conditions. The Laboratory, which will be under the charge of a Senior Lecturer of St. Andrew's University, is presently being extended and caters for routine and for research work.

Applications, with names of three referees, to the Medical Superintendent.

NORTHERN POLYTECHNIC
HOLLOWAY, LONDON, N.7

The Governing Body invites immediate applications for appointment as Lecturer in Inorganic Chemistry. Salary scale, £1,670 by £45 to £1,895 plus London allowance £45/£60.

Apply for further particulars and form of application.

R. H. CURRELL, F.C.A.,
Clerk.

DEPARTMENT OF PHYSICS

UNIVERSITY OF WINDSOR
WINDSOR, ONTARIO, CANADA

Applications are invited for the following staff appointment: Experimental Physicist (Low Energy Nuclear Physics). Preference will be given to candidates with experience in the use and operation of nuclear accelerators. The successful candidate will be expected to instruct in undergraduate and postgraduate courses and to devote a part of his time to research. The appointment may be made at the rank of Assistant Professor, Associate Professor, or Professor, at which a properly qualified candidate may expect a salary of not less than $8,000, $10,000, and $13,500 per annum respectively.

Inquiries should be addressed to Professor L. Krause, Head, Department of Physics.

CHIEF TSETSE OFFICER (MALE)

BECHUANALAND

Required to direct investigations of the ecology of *Glossina morsitans* in the Chobe and Ngamiland districts. Candidates must have a degree in Zoology, entomology or Botany; or a degree in Veterinary Science with experience of Tsetse and Trypanosomiasis problems. Salary, £2,970 per annum, plus 25 per cent terminal gratuity. Passages provided. Quarters available. Education allowances. 2 to 3 year contract.

Please apply for further details, giving full name, qualifications, experience and quoting Ref.: RC 217/19/02 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

OXFORD UNIVERSITY

Electronic Engineer wanted. Proof of extensive experience in pulse and A.C. transistor techniques required. Duties will include maintenance and development of complex existing spectrometric and counting equipment; the development of new devices in sea-going navigation and survey fields as well as other electronic projects in the laboratory. Salary in the range £1,000 to £1,500, depending on experience.—Apply Dr. E. T. Hall, Research Laboratory for Archaeology, 6 Keble Road, Oxford.

IMPERIAL COLLEGE

LONDON, S.W.7

DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY

Assistant Lecturer for research and development in problems at high pressures and temperatures of novel and wide technological interest. Salary £1,050 by £75 to £1,275 per annum, plus F.S.S.U. benefits.

Applications to Professor J. S. Rowlinson.

ANALYST REQUIRED FOR LABORATORY engaged in chemical and emission spectrographic analysis of silicates. Previous experience in this field desirable, but applicants offering other relevant experience considered. Starting salary up to £980 per annum.—Applications should reach the Assistant Bursar (Personnel), University of Reading, Berkshire, as soon as possible.

SCIENTIFIC OFFICER REQUIRED TO work within Department of Ecology at The Grassland Research Institute, Hurley, Maidenhead, Berks, to join team studying the interactions between ruminants and pasture. The appointee will be particularly concerned with the efficiency with which cattle and sheep harvest pasture in various grazing systems and the effect of grazing on the growth of the sward. An interest in both animal and plant is essential and a knowledge of crop physiology desirable. Qualifications: First- or upper second-class honours degree in an appropriate discipline. Initial appointment in Scientific or Senior Scientific Officer grade according to age and experience. Salary scale: S.O. grade, £895 by 14 increments to £1,521; S.S.O. grade, £1,685 by five increments to £2,082.—Apply: Secretary.

JUNIOR TECHNICAL OFFICER REQUIRED for interesting work with radioisotopes in the study for tumour localization in animals. Applicants, aged 22 and over, need Pass Degree or H.N.C. in biology or physics with "A" level in the other subject. Under 22, "A" levels in both subjects. Salary scale in the range £575 to £1,221 including London weighting.—Write with full details to the Director, Medical Research Council, Cyclotron Unit, Hammersmith Hospital, Ducane Road, London, W.12.

UNIVERSITY OF BRISTOL

Applications are invited from graduates for a Research appointment in the Department of Botany for work on the Physiology of insect-pathogenic fungi. The appointment is for one year in the first instance, but may be renewed for a further period. Salary scale either £760 to £840 or £1,050 by £75 to £1,275 according to first or higher qualifications.

Further particulars may be had from the Registrar, University Senate House, Bristol, 2, to whom applications should be sent by November 8.

UNIVERSITY OF WINDSOR

Applications are invited for the following positions in the Faculty of Applied Science.

PROFESSOR AND HEAD OF THE DEPARTMENT OF CIVIL ENGINEERING

The appointment is to be effective September 1, 1966.

ASSOCIATE OR ASSISTANT PROFESSOR OF INDUSTRIAL ENGINEERING

To initiate a new programme at both graduate and undergraduate levels.

Applicants should have the Ph.D. and background in operations research. The appointment is to be effective about July 1, 1966.

Applications and correspondence should be directed to:

Dr. J. Gordon Parr,
Professor of the Faculty of Applied Science,
University of Windsor,
Windsor, Ontario, Canada.

PSYCHIATRIC RESEARCH UNIT

UNIVERSITY HOSPITAL,
SASKATOON, SASKATCHEWAN

Applications are invited from Chemists or Biochemists (with Ph.D.) for a research post. Present research interests of the unit are mainly Chemistry and Biochemistry of the Indoles, Catecholamines, and Aminochromes and their relation to mental illness. Salary in the range of $8,316 to $10,092.

Further details and application forms may be obtained from the Personnel Officer, Sask. Department of Public Health, Public Health Building, Regina, Sask., Canada.

UNIVERSITY OF OXFORD. POST-doctoral biochemist required to work on the metabolism of the lens in relation to eye disease. Salary according to experience.—Apply to Dr. R. van Heyningen, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford.

CLARENDON LABORATORY, UNIVERSITY of Oxford, has an opportunity for a Physicist—preferably but not necessarily a graduate—to carry out measurements on superconducting materials in high magnetic fields. Salary on University scales according to qualifications, age, and experience subject to a maximum of £2,200 per annum. Membership of F.S.S.U., good holidays. —Apply in writing, giving fullest details of education and experience, to Dr. A. J. Croft, Clarendon Laboratory, Parks Road, Oxford.

UNIVERSITY OF OXFORD. DEPARTmental Research Assistant required, graduate in chemistry or holder of H.N.C., to be responsible for the operation of n.m.r. and e.s.r. spectrometers. Previous experience preferred but training can be given. Salary within range £600 to £1,750 according to qualifications and experience. University pension scheme.—Applications, with the names of two referees, to the Secretary, Inorganic Chemistry Laboratory, South Parks Road, Oxford.

LADY GRADUATE IN CHEMISTRY OR equivalent for interesting documentation project and editorial work. Some knowledge of German desirable.—Box No. 867, T. G. Scott and Son, Ltd., 1 Clement's Inn, London, W.C.2.

FELLOWSHIPS AND STUDENTSHIPS

POSTDOCTORAL FELLOWSHIP ($6,000 tax-free stipend) in protein chemistry available early 1966 for applicants with previous experience in this area. Study will involve physicochemical, chemical and enzymatic investigations. —Apply with curriculum vitae and two letters of reference to Dr. C. M. Kay, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

RAMSAY MEMORIAL FELLOWSHIPS FOR CHEMICAL RESEARCH

The Trustees will consider in April, 1966, applications for two Fellowships, normally tenable for two years:

(1) British (General) Fellowship, for post-doctoral research, value in range £1,500 to £2,000 per annum, plus expenses grant of up to £100 per annum. May be superannuable.

(2) Fellowship limited to candidates educated at a Glasgow University, for research normally at Ph.D. level, value £750 per annum, plus expenses grant of up to £150 per annum. Not superannuable.

Full particulars from Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, W.C.1. Completed application forms must be received by January 30, 1966.

UNIVERSITY OF ADELAIDE

WAITE AGRICULTURAL RESEARCH INSTITUTE

DEPARTMENT OF PLANT PATHOLOGY

Applications are invited for the following:

(a) Postdoctoral Fellowship in Virology: This fellowship is available immediately for work on virus problems. Preference will be given to applicants interested in transmission of virus diseases including nematode transmission. Stipend £A1,600 to £A1,800 per annum.

(b) Postdoctoral Fellowship in Forest Pathology: This fellowship will be available from about June, 1966, and is for work in any field of Forest Pathology. Stipend £A1,600 to £A1,800 per annum.

(c) Postgraduate Studentship for Work on Cereal Root Rots: This studentship is available immediately. The student will be expected to give special attention to diseases caused by *Rhizoctonia* and *Ophiobolus*. Stipend £A700 to £A1,200 depending on qualifications and experience.

(d) Postgraduate Studentship: A studentship for work on the association between the fungus *Amylostereum* and the *Sirex* wasp in causing damage to forest trees is available immediately. Stipend £A700 to £A900 depending on qualifications.

The Department of Plant Pathology has an academic staff of nine and an active postgraduate school covering air-borne and soil-borne fungal diseases, virus diseases and nematode diseases. Further information about the Fellowships or Studentships can be obtained from the Head of the Department, Professor N. T. Flentje.

Applications should reach the Registrar of the University at North Terrace, Adelaide, South Australia by December 1, 1965.

UNIVERSITY OF OXFORD

MERTON COLLEGE

FELLOWSHIP IN MATHEMATICS

The College proposes to appoint a Fellow and Tutor in Mathematics, if a suitable candidate presents himself. The person appointed will be especially responsible for the teaching of Applied Mathematics in the College. He will be required to take up his duties not later than October 1, 1966. He will be entitled to free luncheons and dinners and, of unmarried, to free rooms in College. His stipend will be determined according to a scale based on age, for details of which application should be made to the College Secretary.

Applicants should apply to the Warden not later than January 1, 1966, giving a curriculum vitae and naming two or three persons to whom reference can be made. Copies of publications may be sent.

The College will not necessarily confine its choice to those who apply.

COLLEGE OF ADVANCED TECHNOLOGY
BIRMINGHAM

UNIVERSITY OF ASTON IN BIRMINGHAM
designate

DEPARTMENT OF CHEMISTRY

ASSOCIATE RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral fellowship for research into the synthesis of pyridine and N. heterocyclics. The work will be carried out in collaboration with Dr. J. D. Butler. Candidates should possess the degree of Ph.D. or be about to submit a Ph.D. thesis. The salary will be on the scale £1,050 to £1,275 per annum.

Further particulars and forms of application from the Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, to whom applications should be forwarded within fourteen days of this advertisement. Please quote: 435/6.

LECTURES AND COURSES

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY
(Faculty of Technology in the University of Manchester)

A two-day course on
COMBUSTION, FLAME AND EXPLOSION HAZARDS IN GASES
will be held in the College on
January 6 and 7, 1966

Reviews of fundamental aspects of spontaneous ignition, combustion reactions, flame propagation and the transition to detonation will be followed by discussions of inhibitors, dust explosions and industrial safety.

Lectures will be given by Mr. G. K. Adams, Dr. J. H. Burgoyne, Dr. G. Dixon-Lewis, Professor P. Gray, Dr. J. H. Knox, Mr. K. N. Palmer and Dr. R. F. Simmons.

The fee for the course is £6. Accommodation will be available at a reasonable additional charge.

Further particulars can be obtained from the Registrar, The Manchester College of Science and Technology, Manchester 1.

UNIVERSITY OF LONDON: A COURSE of two lectures entitled (1) "The Steric Structure of Medium Ring Compounds and Approaches to Conformational Analysis"; (2) "Conformational Equilibria in Disubstituted Cyclohexanes" at 5.30 p.m. on November 2 and 4 at Westfield College (Large Physics Theatre, Queen's Buildings), Kidderpore Avenue, N.W.3. Admission free, without ticket.—James Henderson, Academic Registrar.

UNIVERSITY COLLEGE LONDON (GOWER St., W.C.1). An Inaugural Lecture by Dr. D. J. Millen, Professor of Chemistry, entitled "Molecular Measurements" will be given on Monday, November 1, at 5.30 p.m.—Admission free, without ticket.

GRANTS & SCHOLARSHIPS

RAMSAY TRAVEL GRANT

The Trustees will consider in April, 1966, applications for a Travel Grant, of up to £350, for 1966-67, limited to junior academic chemistry staff of Universities in Scotland.

Particulars from Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, W.C.1. Completed application forms to be received by January 30, 1966.

PIDA/BSAP TRAVELLING SCHOLARSHIPS

The British Society of Animal Production has available funds provided by the Pig Industry Development Authority to enable both graduates and research workers to study any aspect of pig research in the United Kingdom or abroad. Candidates will be required to indicate the subject they wish to study, provide a detailed itinerary and an estimate of the probable cost.

Application forms are obtainable from the Secretary of the British Society of Animal Production, West of Scotland Agricultural College, Auchincruive, Ayr.

LEVERHULME RESEARCH AWARDS
FELLOWSHIPS AND GRANTS, 1966

Application is invited for Fellowships and Grants-in-aid of research. These awards are intended for senior workers of established position and are limited to British-born subjects normally resident in the United Kingdom; in exceptional circumstances the Trustees may waive the residence condition. No subject of inquiry is excluded from consideration but preference is given to subjects in which existing provision for research is inadequate. The duration of the awards does not extend over more than two years or less than three months and the amount depends on the nature of the research and the circumstances of the applicant.

Application must be made on Form F/2 obtainable, together with further details, from the Secretary, Leverhulme Research Awards, Room 904, 21-23 New Fetter Lane, London, E.C.4. The closing date is December 31, 1965. Results will be announced in May and the awards will normally date from September 1, 1966.

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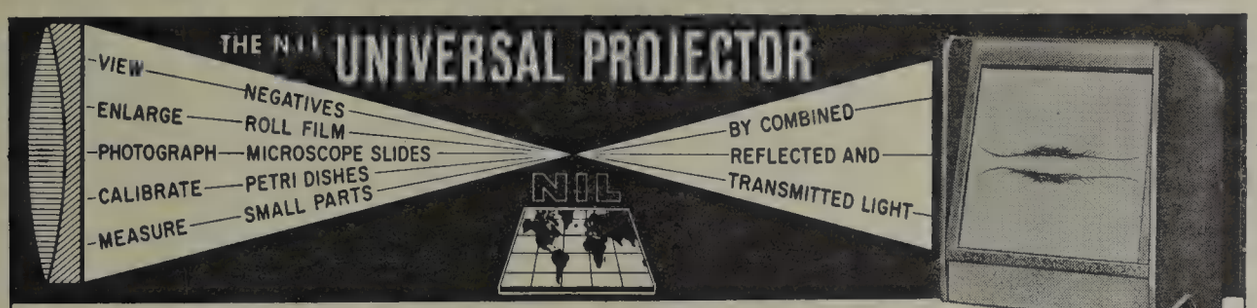
TRADE ANNOUNCEMENTS

PHYSICISTS—"INDELIBADGING" MEANS Jencons will mark indelibly your Pyrex beakers, flasks, volumetric ware and all glassware with the international Radioactive materials symbol at little or no extra charge, also complete radioactive signs service.—Details, Jencons of Hemel Hempstead, Hertfordshire.



DESCRIPTION

The N.I.L. Universal Projector is a table-mounted apparatus, housed in a hammer-tone enamelled cabinet with a clear lacquer overlay. The tilted focussing screen is made of fine-ground optical plate with a non-reflecting surface. Clips hold contact paper for photography. Dimensions: 20" high, 12" deep, and 10" wide. Weight: 13½ lbs.

The 6-volt 5-amp (30 watt) lamp is a point-source, operable continuously without overheating, controlled by a two-position switch.

The specimen platform provides a large surface area (6" x 10"), the condenser-well serving as a pivot and centering control for film and slide accessories. Fine focus is adjustable for all objectives.

FEATURES

- Point-source lamp, highest quality projection lenses, and matching condensers give sharpest enlargement without distortion.
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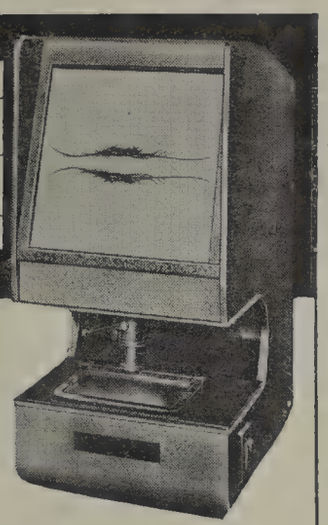

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ACCESSORIES

Focal path extender, 4 different objectives (F80, F50, F35, and F25) and matching condensers, negative holder, roll-film adapter, and slide attachment.

BULLETIN 5-2040

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IN METROPOLITAN WASHINGTON, D. C.
TELEPHONE: (301) 933-1144

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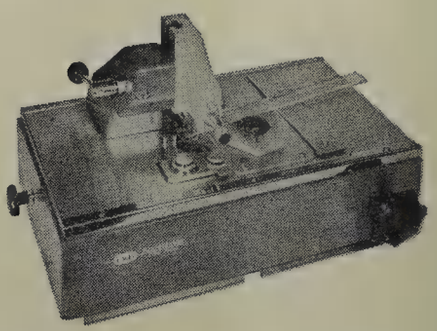

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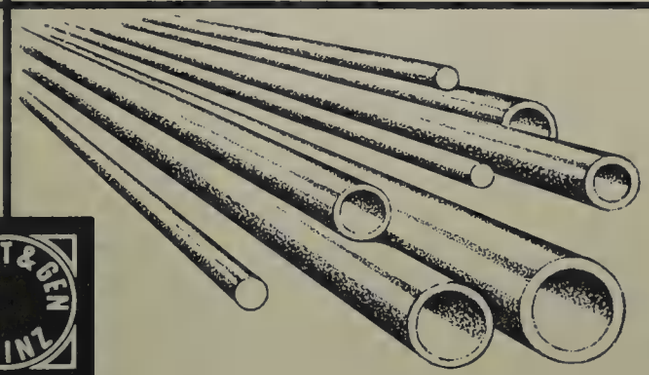

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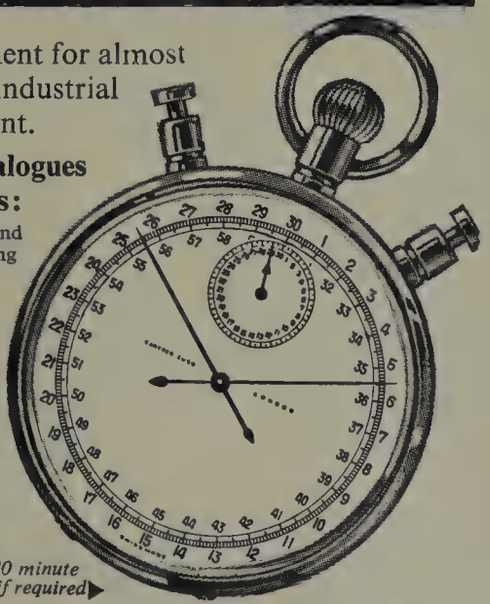

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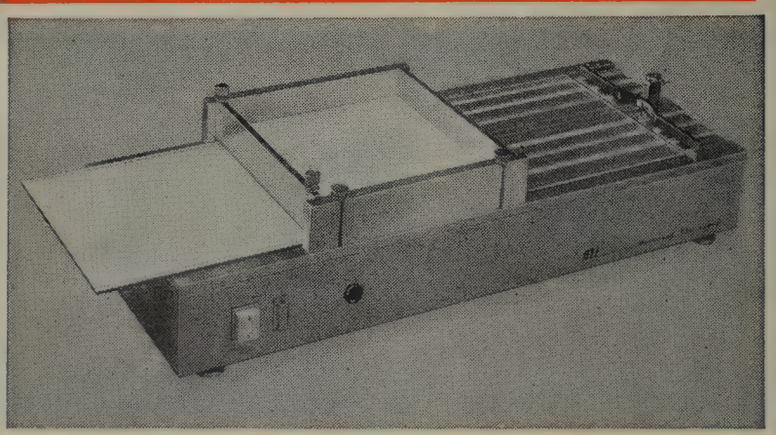

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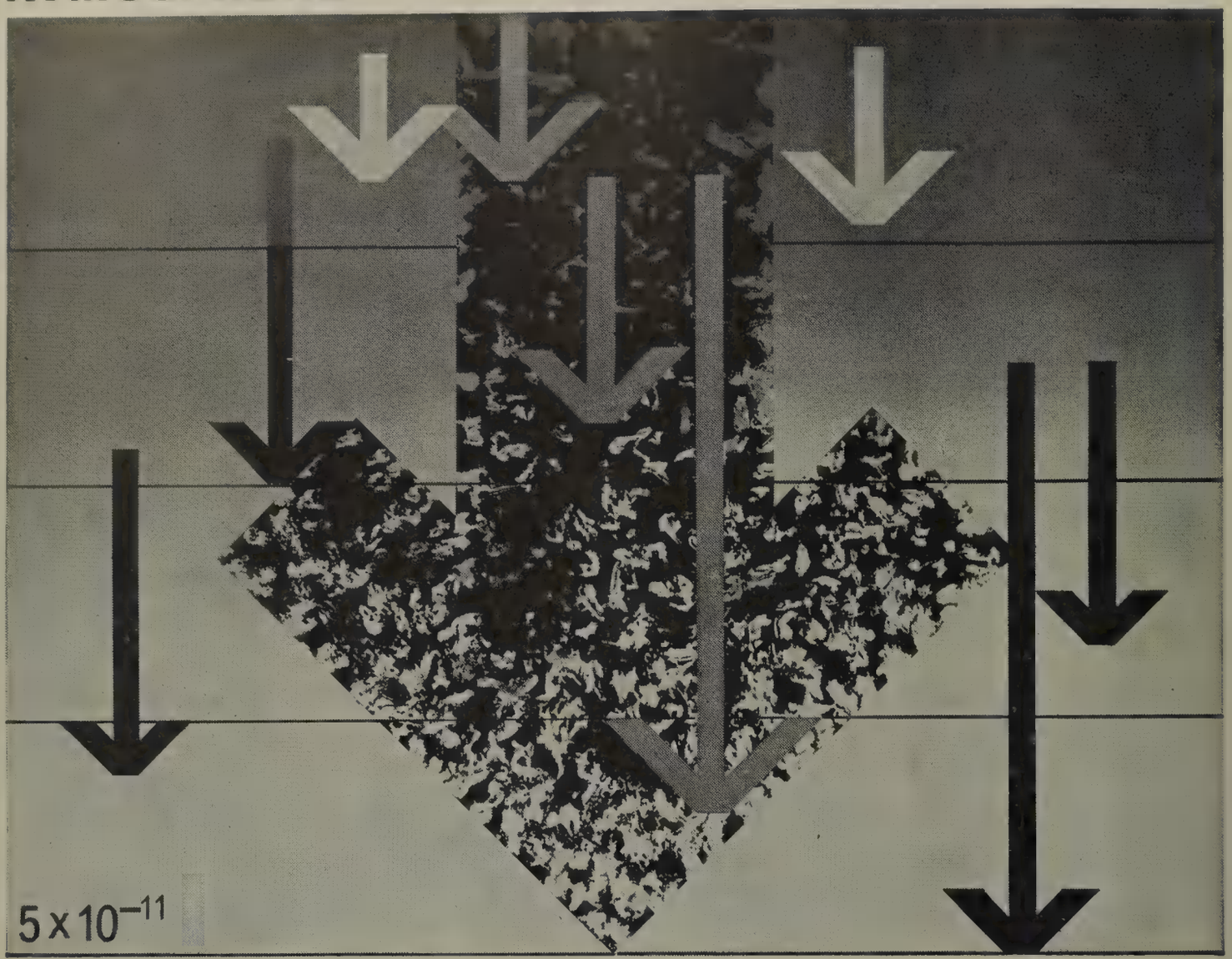

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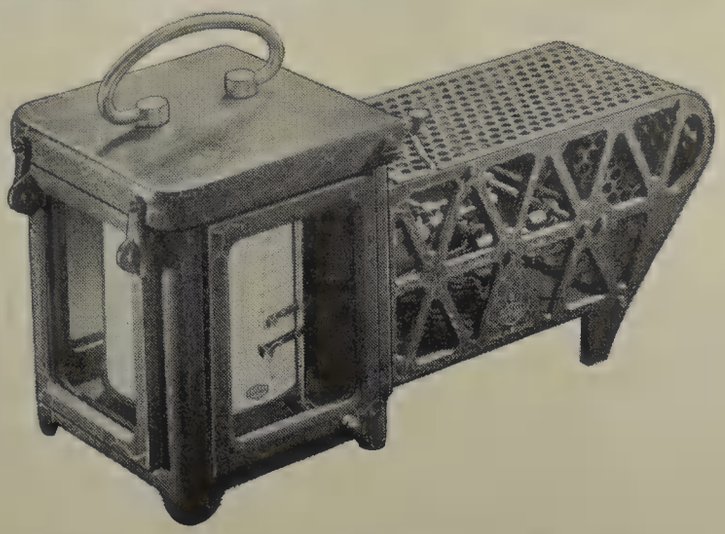

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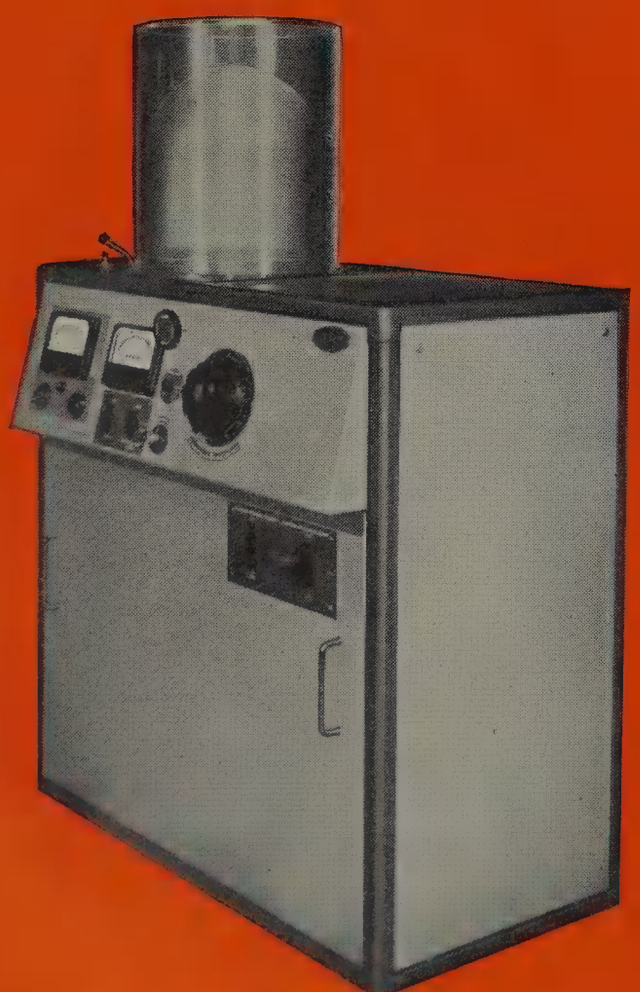

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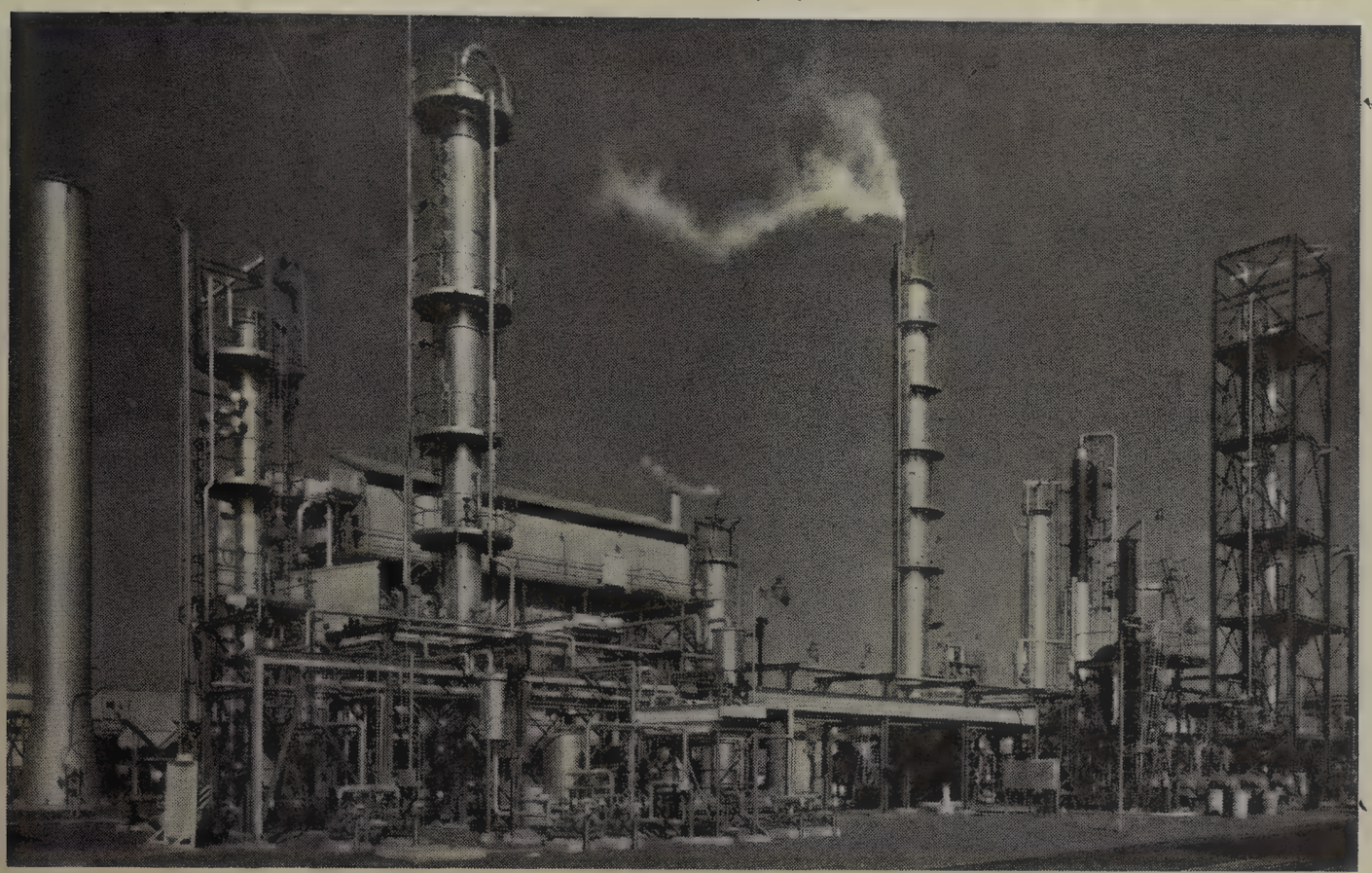

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SCIENTIFIC APPARATUS
BULLETIN NO.7

MALARIA AND THE ELECTRON MICROSCOPE

Fig. 5 *Ookinete of* Plasmodium cynomolgi bustianellii *in the mid-gut of* A. stephensi. *The ookinete is in the process of penetrating the mosquito gut cell. Visible are the anterior slit, the mosquito gut cell and the brush border.* (*Magnification x 31,000*).

Until the introduction of the AEI EM6B, all commercially available electron microscopes were general purpose instruments rather better adapted to metallurgical and similar industrial applications than to biology. This mattered little so long as microbiologists were mainly concerned with morphological problems, but it became a disadvantage when interest shifted to the structure of viruses because it was not possible to reconcile versatility with the highest possible resolution. Now the biologist has in the EM6B an instrument custom-built to his particular needs. It is therefore an appropriate juncture at which to review the ways in which electron microscopy has contributed to the study of small organisms. Consider, therefore, its use in the study of malaria.

Blood forms

The electron microscope was first used in connection with malaria research in 1942 when Wolpers applied it to the study of blood forms. This initial essay did not prove wholly successful because the thickness of the thinnest sections obtainable with the techniques then available was too great for detailed observation. In due course, however, the method of cutting thin sections by means of the ultra-microtome was developed, and the electron microscope began to yield results.

In 1956 Fulton and Flewett proved that the trophozoite stages of *Plasmodium berghei* and *P. knowlesi* lie, not on the periphery of the ethrocytes but inside. This together with the work carried out by Garnham, Bird and Baker on *Lankesterella garnhami*, and by Maria Rudzinska and Trager on the presence of phagotrophic food vacuoles and mitochondria in *P. berghei* and *P. lophurae*, firmly established electron microscopy as a major technique for advanced work in this field.

Observation of sporozoites

The work of Garnham, Bird and Baker was particularly important since it led, in 1960, to the series of papers describing the fine structure of various malaria parasites. Three of these papers deal with forms of the parasite which are pathogenic to both avians and primates as they appear in the sporozoite stage. This is the long thin form of the organism which is injected into the bloodstream of the victim, via the salivary glands of the mosquito. Fig. 1 shows a longitudinal salivary gland containing many sporozoites in the acinar cells and just emerging from them. The sporozoite measures approximately 10 microns long and about 1 micron in width, these measurements varying slightly from one species to another.

In order to examine such specimens in the electron microscope an elaborate preparation technique must be used. After dissection from the mosquito, the specimens are immediately fixed in 1% osmium tetroxide solution buffered to pH 7. After fixation, the tissues are rapidly dehydrated through graded ethanol solutions up to absolute ethanol. 1% phosphotungistic acid is included in the third absolute ethanol change to stain the tissues, before a final rinse in ethanol.

The specimens are then embedded in Araldite, and sectioned on an ultra-microtome (each section being between 60 and 150 Angstroms in thickness) and subsequently examined in the electron microscope.

Constant features

Figures 2, 3 and 4 show typical sporozoites. *Haemameoba gallinacea* is responsible for malaria in birds—i.e. an avian form—while *Laverania falcipara* and *P. vivax* are both

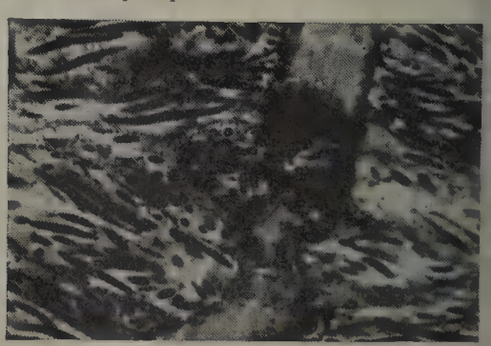

Fig. 1. *Longitudinal section of part of a salivary gland of* Anopheles gambiae *containing a large number of sporozoites of* Laverania falcipara *in the acinar cells and emerging into the salivary duct.* (*x 1,300*).

Fig. 2. *Sporozoite of* Haemamoeba gallinacea *in the salivary glands of* Aedes aegypti queenslandensis. *The longitudinal section is of the middle region of the sporozoite and shows the nucleus, mitochondria and one lobe of the paired organelle. A transverse section also appears showing several mitochondria.* (*x 20,000*).

pathogenic to primates. All three sporozoites show the same basic structure, but minor differences have been questioned. The problems thus raised can only be resolved by electron microscopy.

Constant features are the nucleus, mitochondria, micropyle and thick pellicle. The micropyle is of particular interest since it is now thought to be a characteristic feature of the sporozoites. The electron microscope has shown it to be a constant feature of all sporozoites examined to date, and it has also been discerned in the sporozoan parasite *L. garnhami*. This account, brief though it is, clearly indicates the way in which the electron microscope can be used in the identification of organisms. And since fine structural details seem likely to prove the only specific differences between organisms of the kind under discussion the technique is an important one.

The basic principle is simple. Once constancy of appearance has been demonstrated it may then be used as a positive index in the identification of a given organism. Similarly, its absence is an indication that the organism does not belong to that genus. A comparable exercise has been carried out on the ookinetes of *Haemamoeba* and *Plasmodium*. In this case the specimens were stained in tungstophosphoric acid. A

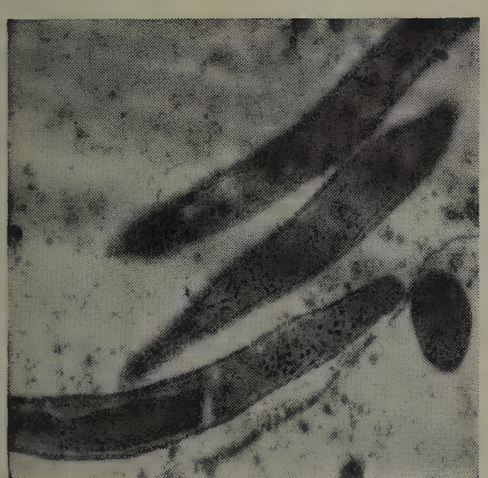

Fig. 3. *Sporozoites of* L. falcipara *in the salivary glands of* A. gambiae. *The sections are longitudinal, a single transverse section being also present, and show the nucleus apparently connected to the micropyle by a granular process and mitochondria.* (*x 10,000*).

few minutes after the mosquito has ingested malaria infected blood, the micro and macrogamete fuse and produce the zygote. This body uncoils and lengthens to become the ookinete.

The ookinete *H. gallinacae* has been studied in detail by Garnham, Bird and Baker. Under the optical microscope the ookinete is seen as a body about 15 microns in length, with a large deeply staining nucleus (often eccentric in position), pigment granules and a vacuole. The appearance under the electron microscope is shown in fig. 5. The nucleus has a patchy granular structure with no clearly defined nuclear membrane. The pigment granules are shown to be in the cytoplasm, appearing as irregular black masses lying in vacuoles limited by a membrane.

After developing for about 24 hours the ookinete is ready to pass through the mucosa of the mosquito's midgut. The process whereby this occurred was not discernable under the optical microscope and it was only comprehended when the electron microscope was applied to the problem.

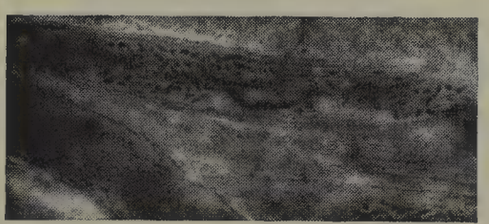

Fig. 4. *Sporozoites of* Plasmodium vivax *in the salivary glands of* A. stephensi. *It shows the paired organelle, micropyle, peripheral fibrils, convoluted tubules and the pellicle.* (*x 19,000*).

Biochemical studies

The fine structural details revealed by the electron microscope raised important questions as to the functions of the various organisms. Consequently research workers are becoming increasingly concerned with biochemical studies involving advanced techniques—such as enzyme localisation by radioactive marking—which call for very high resolution microscopy. The ultra high resolution of the EM6B Electron microscope makes it particularly suitable for work of this kind. It also simplifies more routine biological work, and for a number of reasons.

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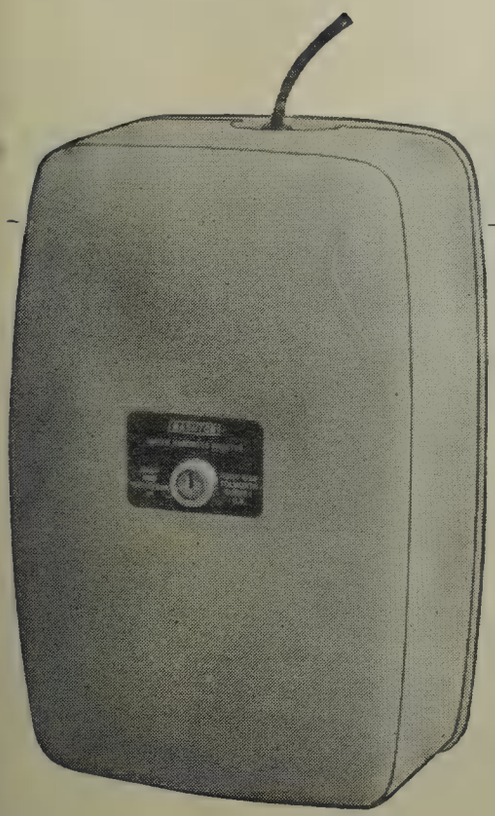

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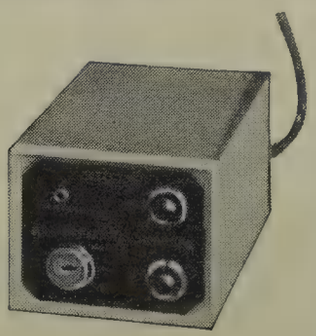

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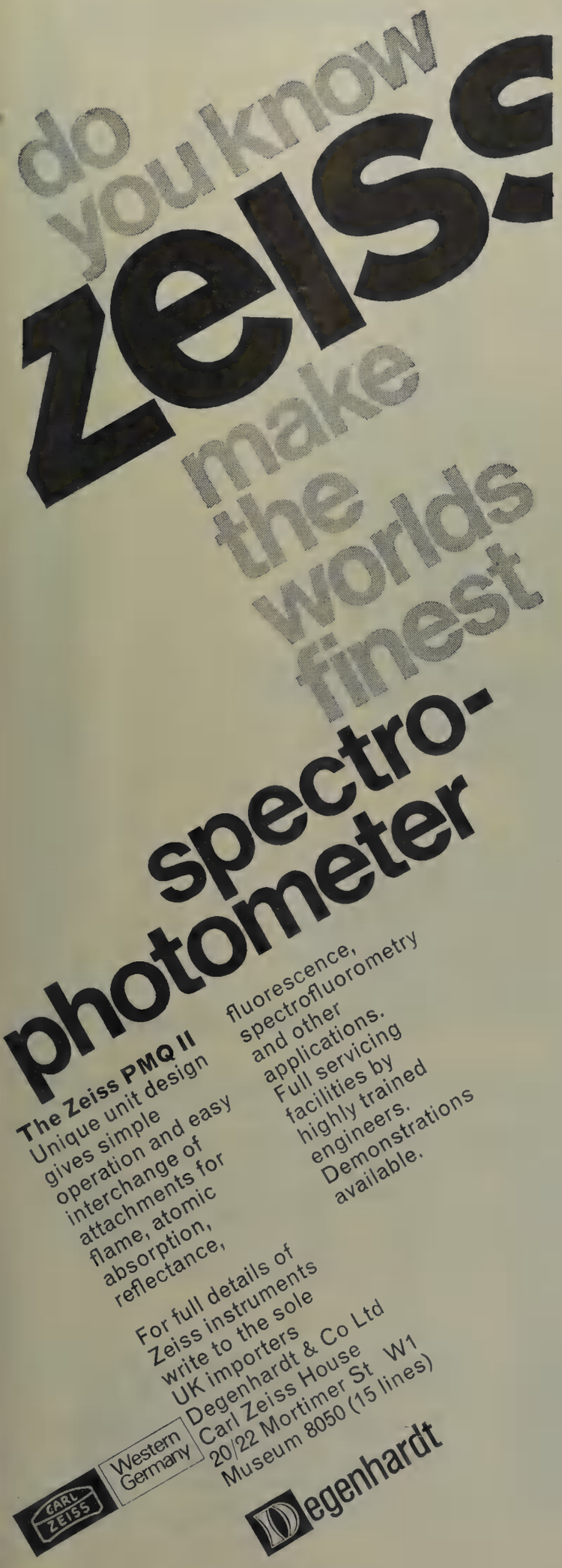

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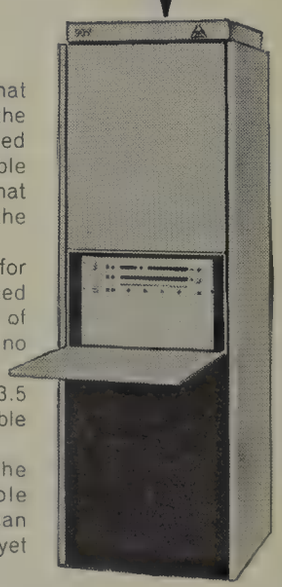

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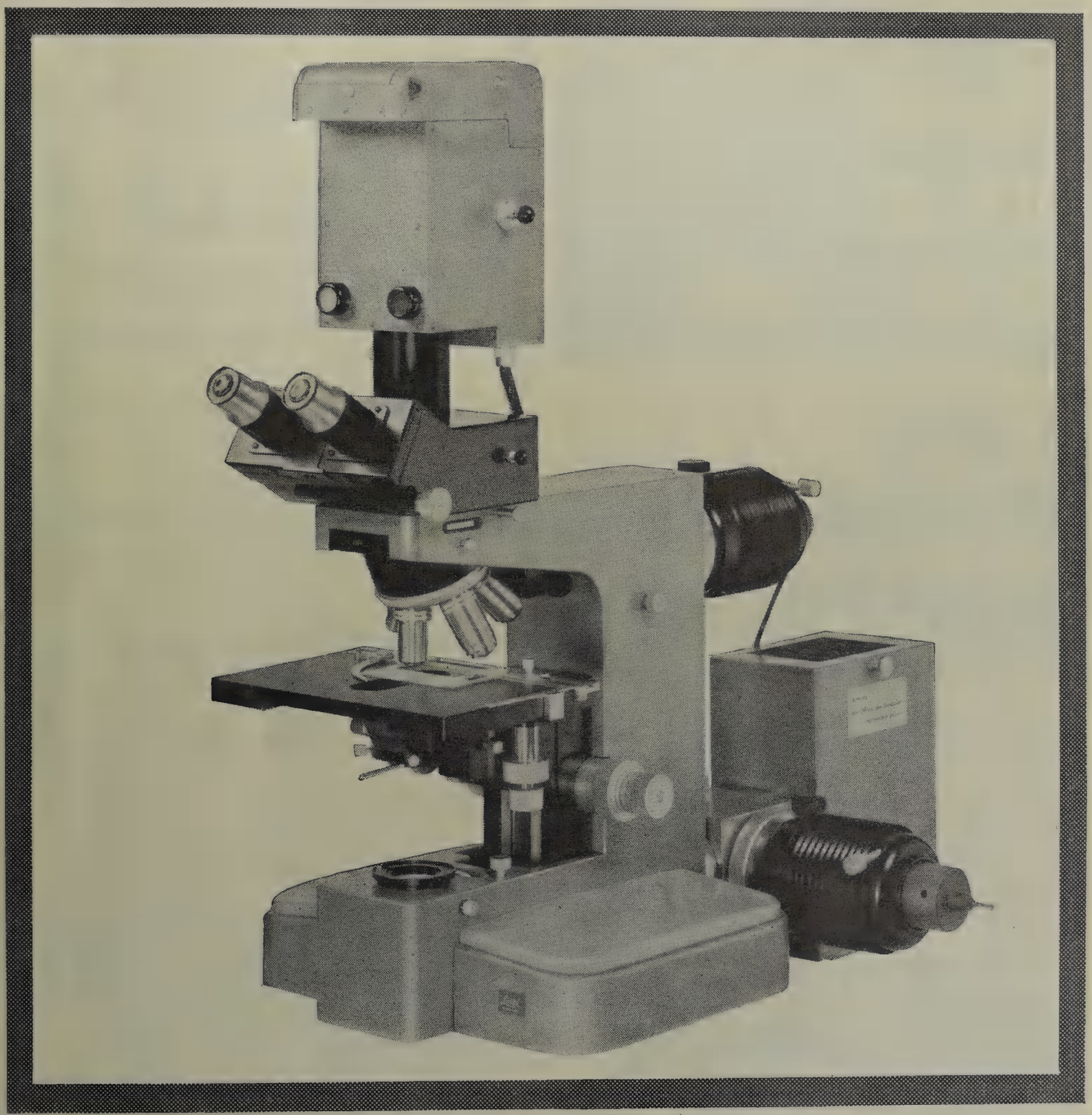

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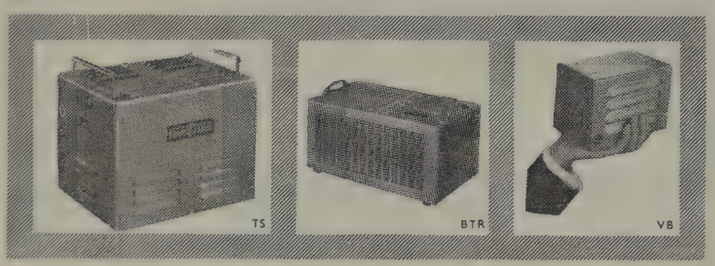

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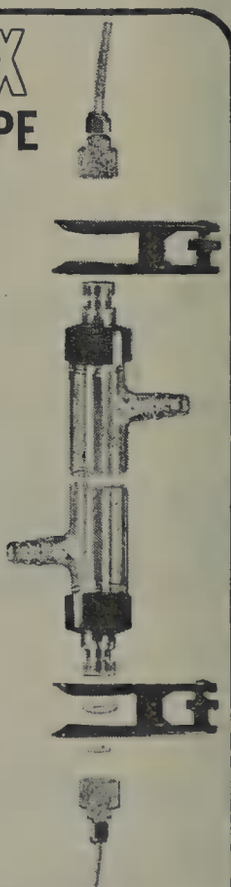

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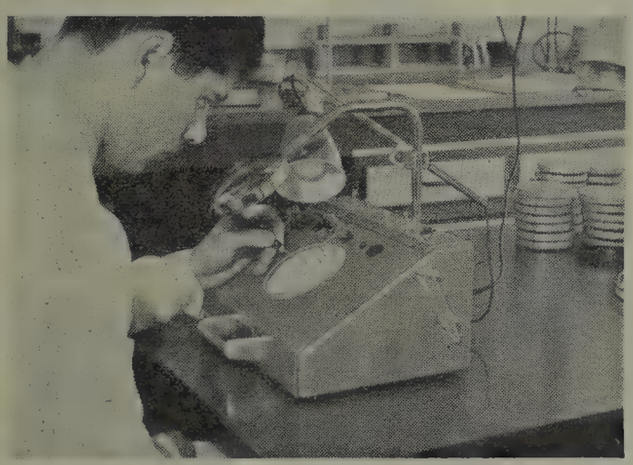

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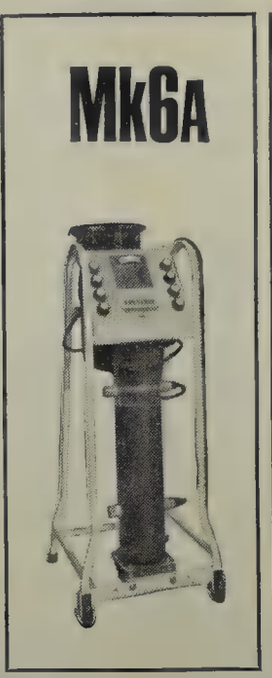

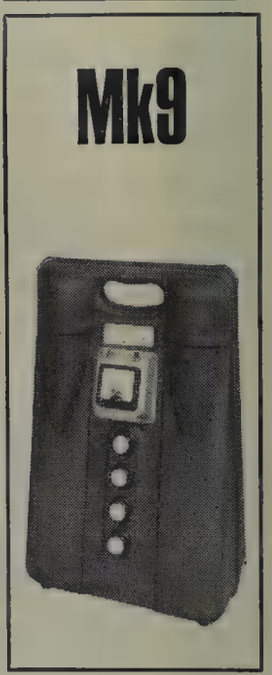

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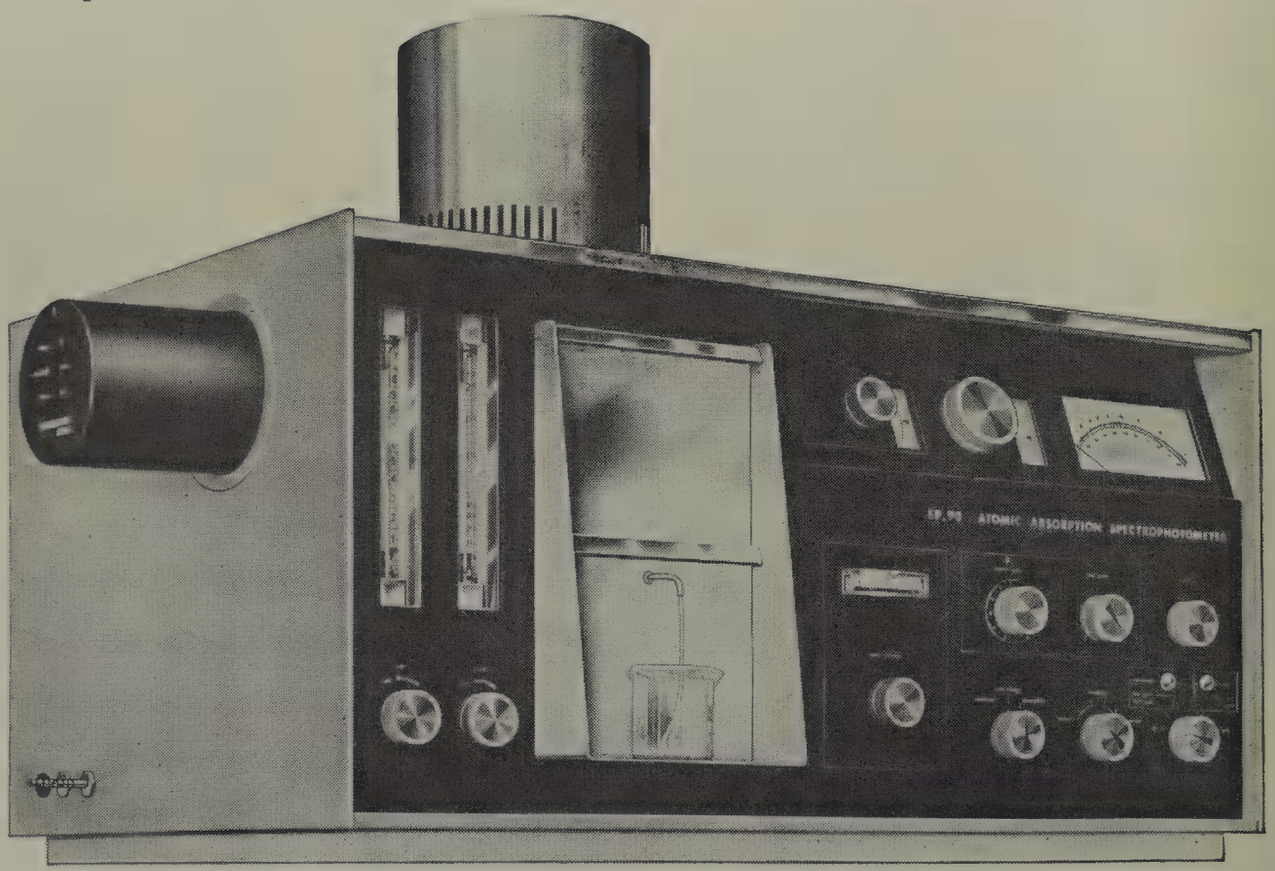

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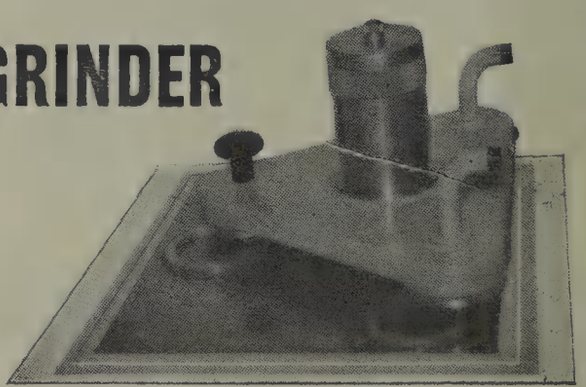

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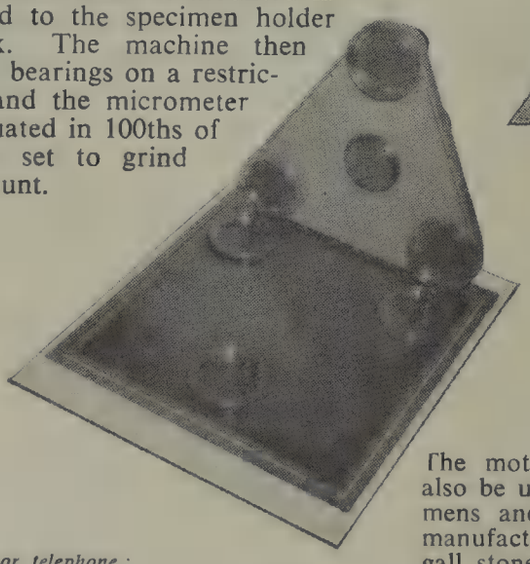

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RETHINKING CIVIL SERVICE POLICY

TO the scientist and technologist one of the most interesting features of the sixth report from the Estimates Committee for the Session 1964–65*, dealing with recruitment to the Civil Service, is the section which considers the needs of the Civil Service not simply for administrators but for specialists and their role in the Service. This is seen not merely through the eyes of the Civil Service itself in the person of Sir Laurence Helsby, head of the Home Civil Service, Sir William Armstrong, Joint Permanent Secretary to the Treasury, and Sir Richard Way, Permanent Secretary to the Ministry of Aviation, for example, but also through those of outside witnesses, including Prof. W. J. M. Mackenzie, Mr. D. N. Chester, Mr. B. J. Holloway and the Institution of Professional Civil Servants. As the Estimates Committee observes, the recruiting methods used depend as much on the assumptions and attitudes about the kind of people required in the Service, the approach to their work which would be required of them, and the division of functions proper within the Service, as on the size and character of the total employment market. It was, therefore, concerned to explore these assumptions and attitudes since they determine the appearance and mode of operation of the Civil Service, both as it is to-day and as it will be during the next generation.

Sir Laurence Helsby believes that there would continue to be a small administrative class, remaining separate from the general service class responsible for the day-to-day run of administration and responsible for somewhat specialized functions which seldom had any precise counterpart in outside organizations—at least below board-level. Staffing at this level called for a particular type of man and for qualities which, he believed, could not be effectively shown in an advertisement, but he did not think that this approach led to a Service which was opposed to change. Sir William Armstrong expressed a similar view and, in regard to mobility, thought it was unlikely to reach a point when, at the end of his life, a man could say, "I have spent as much time out of the Service as in it". The distinction between the man whose basic career is the Civil Service but who goes out from time to time and the man who is really temporary would remain. He did not deny that there was any need for a reappraisal of the pattern of the Civil Service, and Sir Laurence Helsby suggested that it would be well to wait at least a year before making a broader enquiry into the organization, recruiting and structure of the Civil Service. However, he did agree with the suggestion that such a review would be desirable in spite of the probability that developments in the structure of Government would be rapid for ten years to come.

Sir William Armstrong directed attention to the limitations of the use of techniques such as cost–benefit analysis which were placed by the number of people familiar with these techniques both in the departments concerned and elsewhere in the Government service, local authorities and the nationalized industries. This is accordingly a question of the special trained manpower, and Sir William maintained that the really pressing need was for more specialists, particularly as economic advisers—though in the long term it was probable that the Service would develop its own brand of expert in what might be called 'Government economics'. Here the Centre for Administrative Studies might help, but other witnesses saw a danger of rigidity in such a development.

Sir Richard Way and Sir Eric Roll, the Permanent Under-Secretary of State at the Department of Economic Affairs, made some interesting observations on the part played by the scientist and other specialists and their relation to administrators. Sir Richard was emphatic that a scientist equipped to do administrative work would not be deterred from entering the scientific grade by the prospects of promotion. However, he thought that for the position of scientific officer the Service required a man competent for a particular piece of research, without special personal qualities, whereas the administrative class Civil Servant in the higher grades should have had wide experience. Scientists destined for more senior posts were helped to widen their experience while remaining specialists. When Sir Richard had encouraged scientists to enter the administrative class he had found that they had usually wished to remain where they were; in his view it was a wholly false picture to imagine that every scientist wanted to become an administrator. The distinction between administrators and scientists was not a hindrance to recruiting. On the contrary, many young scientists who entered the Service would be deterred by the abolition of this distinction. Nor did he think that scientists were deterred by the thought that administrators were amateurs; rather the scientist would regard the administrator as being highly professional in the field he had chosen.

Sir Eric Roll suggested that the relation of economists to administrators was rather different simply because the tasks of Government had a much greater affinity with the field of interest of economists and other social scientists. Nevertheless, though the division between economists and administrators was not so easily defined as that between nuclear physicists and biochemists and administrators, the tasks of the administrator differed in kind from those of the economist. Pointing out that 43 per cent of the administrators in the Department of Economic Affairs had degrees or other qualifications in economics, Sir Eric added that no difficulties from rigid procedures had arisen in obtaining specialized staff. Lack of flexibility had had no real effect on the Department's recruitment possibilities.

These views, however, were not unchallenged in the evidence. The memorandum from the Institution of Professional Civil Servants expressed the unanimous view that if the professional and similar specialists classes were to be given managerial responsibility and if this were to become known, it would have an important and beneficial effect on recruitment. The professional classes, at present, have a strong feeling that they are not given full and proper scope to discharge the responsibilities that they are equipped to carry. Even propaganda in the schools would be more effective if it could be shown that specialists in general were not at a disadvantage in terms of responsibility and advancement to the highest posts. The evidence of the Ministry of Aviation in regard to the transfer of scientists to the administrative class was questioned as inaccurate. It was pointed out that experience in industry shows that transfers to broaden

* Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964–65—Recruitment to the Civil Service. Pp. xxxviii+269. (London: H.M.S.O., 1965.) 26s. net. (See also *Nature*, **208**, 311; 1965.)

experience are neither unpopular among young scientists nor a deterrent to recruitment. Mr. D. N. Chester, however, suggests that the evidence indicates that science graduates with first-class degrees prefer to continue with scientific work rather than take administrative or managerial posts. Certainly, he doubts whether this will change when the output of scientists is greatly increased. Nor has it yet been proved that those with a scientific training have a particular aptitude for the higher levels of administration.

The Institution of Professional Civil Servants submits that for the specialist Civil Service the Government's employment policy should rest on three main principles. It should exploit to the full the training and talents of its scientists, technologists and technicians and ensure that there is full employment in terms of the quality of work they are given to do. It should make clear that specialists are full and equal partners with other staff, and that they have an equal opportunity to promotion to the highest posts. It should offer pay and careers which demonstrate that scientists, technologists and technicians are no longer regarded as inferior or second-rate.

In all this evidence there is little that is not to be found in the Barlow Report on scientific staff which was released almost twenty years ago. The Estimates Committee comments that the picture which emerges from this examination is one of considerable development in the patterns and traditions of the Civil Service and of readiness for further development—basically, however, development within an established framework of assumptions. There does not appear to have been any fundamental questioning of the basic structure, and the Estimates Committee finds it hard to accept that the task of Government justifies the unique significance attaching to the administrative class and that only a select few are fitted to undertake this work. It also questions the assumption that all a specialist wants to do on leaving university is to work within his specialized subject. In this connexion, the Committee directs attention to the evidence of Mr. P. F. Nind, of the Shell International Petroleum Co., Ltd., that relatively few among specialist technical staff desire promotion in their own narrow function, whereas many wish to broaden their experience and run small companies elsewhere. This, as Mr. Nind remarked, is entering general management.

While the Estimates Committee welcomes the changes that have already been made or are planned to increase the flexibility of the Civil Service and its ability to undertake new tasks, it considers, nevertheless, that this aspect needs further and much more systematic study. Accordingly, this is one of the considerations which could well be subjected to further enquiry—in particular, the actual tasks undertaken and those that should be undertaken by the administrative class in the near future. The Committee discusses, moreover, recruitment to the Scientific Civil Service in a further section of its report, and here there are some observations that throw further light on the evidence given by Mr. Nind.

The experience of the Civil Service has been that young scientists prefer to become members of a particular team or establishment, rather than to join the Scientific Civil Service as such. This was confirmed by Mr. B. J. Holloway, secretary of the Appointments Board of the University of Manchester, who in expressing the view that the Scientific Civil Service was the section of the Civil Service which had really faced the problems of competitive recruitment—a view which the Estimates Committee endorsed—remarked that to the fresh graduate it was a more stimulating thought to work, say, at Farnborough than to apply to the Civil Service Commission for establishment as a scientific officer. Nevertheless, although Sir Richard Way fully recognized that establishments like Farnborough and Malvern are far more vital and attractive than the Ministry of Aviation as such, his other observations indicate how far some of the senior officers in the Civil Service still are from understanding the scientific life and even the implications of Sir Alan Barlow's Report.

Rejecting the suggestion that older scientists who had passed their creative years might be transferred to administration, Sir Richard characterized as absurd "the idea that a worn-out scientist can, as an afterthought, come in as an administrator". This is a complete misunderstanding, if not misrepresentation, of how to use to the maximum public advantage the skill and experience of trained manpower. The large industrial firms have long recognized that in research, as in art, creative powers are at their peak in youth but tend to decline in middle life; experience, on the other hand, grows and may be used to advantage in many ways. It is here that the Civil Service appears to have fallen behind industrial and commercial practice, for example, and this may well contribute to the unsatisfactory overall situation for scientists, though the general shortage of good graduates and competition are clearly contributory factors. It may be noted that Sir Laurence Helsby in his evidence admitted that mobility in the Service was not so high as he would like it to be, and was clearly not opposed to the use of scientists in administration. The Estimates Committee concludes this section of its report with the remark that, quite apart from the importance of providing good promotion prospects for the scientist, the man with the scientific or mathematical background was more than ever needed in management to-day, in view of the importance of the ability to quantify problems: this is at least as true of public administration.

In discussing recruitment for modern needs, in the final section of its report, the Estimates Committee draws together the evidence which impressed it most with the need for a thorough examination of the problems of recruitment to the Civil Service. All the evidence shows that the Civil Service and the Civil Service Commission are aware that not all the requirements of the Service are being met and that efforts are being made to meet them. Nevertheless, the Estimates Committee believes that the measures taken have not yet been extended to a re-examination of the structure of the Service and other issues underlying recruitment. In three areas in particular detailed examination might be expected to lead to proposals for change.

The first is in the extent of mobility, both within the Service and between the Service and other occupations, including consideration of other means of acquiring wider experience and keeping the Service in touch with the developing community. The second is the need for professional training and the means available for acquiring a knowledge of specialist techniques at the beginning and in the course of a career. The third is the urgent need to examine the supply of qualified manpower on which the Civil Service is to draw. It is a fallacy, as the Estimates Committee remarks, to suppose that, in the long term, the supply of qualified people in any one field is fixed, because the recruiting policies of the Government inevitably affect the total supply. However, in these last two areas, it can be added that enquiry could lead to suggestions for the wise and efficient use of trained manpower,

such as are implicit, though not explicit, in the memorand a from the Institution of Professional Civil Servants.

Research and public discussion on theso important developments could contribute to what Prof. W. J. M. Mackenzie termed "the shaping of a public doctrine about the place of the Civil Service in modern Britain". This, he thought, would be a most valuable result of the appointment of a Royal Commission such as he had suggested at the end of his evidence. This Commission should be a relatively small one, of independent standing, empowered to recruit its own research staff, and not placed, as was the Priestley Commission, in a situation which demanded an urgent and specific report. Mr. D. N. Chester also supported the appointment of a Royal Commission, provided a suitable chairman and membership could be found, and the Commission was adequately staffed and empowered to arrange for research. He suggested that it should be established in 1967, thus giving adequate time for preparatory work and discussion.

This date accords with the views expressed in evidence by Sir William Armstrong and Sir Laurence Helsby; the latter, however, suggested that there was great advantage in having a review of the Service undertaken by a group of people, some of whom, in any event, had had direct experience of its working. Moreover, such an internal committee of officials would have access to information which could not easily be made available even to a Royal Commission. A Royal Commission, however, has advantages in authority and in the publicity in which its enquiries are usually conducted. The Estimates Committee was impressed by the desirability of a preliminary internal enquiry and also emphasizes the value of well-directed and independent research. Its recommendatio ns appear to be in accordance with the balance of the evidence. They are: (1) appointment of a committee of officials, aided by members from outside the Civil Service on the lines of the Plowden Committee, to initiate research on, to examine and report on the structure, recruitment and management of the Civil Service; (2) that the Government should review the position immediately on receipt of the report, and report to Parliament the action it proposes to take, appointing forthwith a Royal Commission if further enquiry by such a Commission is then found to be necessary. Meanwhile the Estimates Committee's report should stimulate preliminary discussion and the Parliamentary and Scientific Committee should be able to ensure that the whole question is not side-tracked, whether on pretext of Parliamentary time or any other excuse.

DICTIONARY OF ORGANIC COMPOUNDS

Dictionary of Organic Compounds

The Constitution and Physical, Chemical and other Properties of the Principal Carbon Compounds and Their Derivatives, together with Relevant Literature References. Edited by Dr. J. R. A. Pollock and Dr. R. Stevens. Fourth, completely revised, enlarged and re-set edition. Vol. 1: A–Chlop. Pp. xxx + 1–588. Vol. 2: Chlor–Dim. Pp. 589–1230. Vol. 3: Din–Iza. Pp. 1231–1976. Vol. 4: J–Phloi. Pp. 1977–2736. Vol. 5: Phlor–Zym. Pp. 2737–3282. First Supplement 1965. Pp. 207. (London: Eyre and Spottiswoode (Publishers), Ltd.; and E. and F. N. Spon, Ltd., 1965.) £100 the set.

THE *Dictionary of Organic Compounds* requires no introduction to organic chemists, to whom it is always known as "Heilbron's Dictionary", for the late Sir Ian Heilbron was the moving spirit at its initiation and guided it vigorously through its subsequent development until well into the preparation of the present edition.

Workers in neighbouring sciences who may occasionally require information about specific organic compounds may, however, welcome a brief indication of its nature and contents. The first edition appeared during 1934–1937 in three volumes, to be followed during 1943–1953 by the second and third editions, the latter appearing in four volumes. The editors of the first edition accepted the formidable task of selecting from the vast number of recorded organic compounds those to which they considered organic chemists would most frequently want to refer. These were listed in alphabetical order, with their molecular formulae, structural formulae when desirable, and physical properties: this information was followed by a list of the main derivatives of the compound, and a final list of original references. This pattern of the *Dictionary* remains unchanged. The volumes, therefore, provide a rapid and reasonably up-to-date reference to a very large number of compounds. Information about other organic compounds may be sought either in *Beilstein's Handbuch*, which by virtue of its size labours along several years out of date but provides exhaustive treatment, or in the many indexes, annual, five-yearly and decennial, of *Chemical Abstracts*.

The new fourth edition has been completely revised, enlarged to five volumes and very handsomely re-set and bound: the type now adopted gives a very clear and attractive appearance to the pages. The edition includes material obtained by searching *Chemical Abstracts* up to 1962, and by a review of the major chemical journals up to the end of 1963. It is stated to "include all major natural products, pharmaceuticals, pesticides and similar groups of applied chemicals"; natural products, therefore, now appear prominently. It is accompanied by a supplement as a separate volume, which contains new material published in and before 1964. This supplement represents a new feature, for it is the first of a series of annual supplements, of which the fifth will be a cumulative edition containing the material of that year and of the preceding four years: the annual supplements will then continue until the tenth number, which will again be a cumulative volume containing the material of the immediate past five years. (Librarians should note that there is a subscription scheme available for the purchase of these supplements under which the cumulative volumes can be purchased at half-price.)

This fourth edition is about 40 per cent larger than the third, and the considerable increase in the number of entries has largely coincided with various changes of nomenclature: since the names of the compounds form their own index, the editors had to ensure that any compound could be readily found. Many compounds have trivial or common names which are very widely used: in such cases the editors have used their discretion in deciding whether the compound shall be entered in detail under its trivial or its systematic name; since in almost all such cases cross-references (each in its appropriate alphabetical position) are given, no difficulty should arise. Drugs and pesticides in this respect fall into a special category. Since almost all modern drugs, including antibiotics, are known to manufacturers, to the medical profession and to pharmacists and chemists by trade-names (and systematic names would be exceedingly cumbersome), the *Dictionary* uses the names approved by the British Pharmaceutical Commission of the General Medical Council. Similarly, pesticides (although often of more simple structure) are entered under the common names recommended in the *British Standards List* B.S. 1931 (1961). Many proteins fortunately now have structure 'names' based on generally accepted conventional abbreviations.

The main difficulty arises from the fact that many systematic names, and also the numbering of (in par-

ticular) many heterocyclic systems, have been changed from time to time by the nomenclature committees of the International Union of Pure and Applied Chemistry; the last set of rules (now fairly widely accepted) were published in 1957. The introduction to Volume 1 summarizes "a few of the outstanding basic rules of nomenclature from the I.U.P.A.C. 1957 rules" in 8½ pages. These rules are, however, often complex and detailed, and cannot be readily understood and applied without ample structural formulae and examples for illustration. The space given in the introduction is insufficient for this purpose, but a reasonably full account would clearly occupy far too much space. The reader could well be referred to the *Handbook for Chemical Authors* (Chemical Society Special Publication No. 14, 1960) by Dr. R. S. Cahn and Dr. L. C. Cross, where the 1957 rules are explained and discussed in detail. It also contains the highly specialized rules agreed in 1952 between the committees of the Chemical Society and the American Chemical Society for organophosphorus compounds, an increasingly common group of compounds to which the *Dictionary* gives little attention.

Two points regarding the presentation may be made. It has already been stated that under the name of the 'parent' compound is a list of its chief derivatives and also a list of references. Now it often happens that a reader is consulting the *Dictionary* to obtain information on one specific derivative: the *Dictionary* usually provides for solid derivatives the melting-point and the solvent for crystallization, and the reader, requiring more information, is given no guidance as to which of the references record this compound, and he has therefore to work steadily and laboriously through them. For example, under "Phenylacetic acid" (p. 2666) there are nearly thirty derivatives listed, followed by eleven references in various journals: the reader who ultimately finds the required information in the eleventh reference will be weary and rather exasperated. This disadvantage could have been overcome, for example, by placing small superscript letters alphabetically above each reference: the names of the derivatives would then each carry the superscript letter of the reference in which they are to be found. This could easily have been done in the early days of the *Dictionary*, but would now involve much tedious work.

The second point, of lesser importance, is that the elements in the molecular formulae "are given in the order C,H,O,N,Cl,Br,F,S,P with any others following in alphabetical order" (Volume 1, p. xi). This is quite clear, but slightly old-fashioned. The modern formulae indexes of *Beilstein*, *Chemical Abstracts*, etc., all adopt the more simple system of giving the elements in the order C,H, with all the remainder following in alphabetical order.

One minor point. One solvent used for crystallization is frequently named 'petroleum ether' or 'pet. ether'. This term is a relic of the old days when a set of solvent bottles bearing permanent titles normally included 'sulphuric ether' and 'petroleum ether'. The first title has long been obsolete and the second should rapidly have followed it: certainly no organic chemist should call a hydrocarbon an ether. The *Dictionary* also uses the rather old-fashioned alternative 'ligroin': the use of 'petroleum', possibly abbreviated to 'petrol', throughout would surely have been preferable?

These points, however, do not seriously detract from the very great value of the immense amount of classified information which this fourth edition contains—information which with the Supplement 1965 is based on more than 40,000 entries. The heavy labours of the editorial staff, moreover, receive deservedly excellent presentation at the hands of the printers. Had Sir Ian Heilbron survived, this edition would certainly have been a source of great pride to him.

F. G. Mann

W. M. W. HAFFKINE

The Brilliant and Tragic Life of W. M. W. Haffkine

Bacteriologist. By Selman A. Waksman. Pp. 87 + 3 plates. (New Brunswick, N.J.: Rutgers University Press, 1964.) 3.75 dollars.

THE author of this book visited the Haffkine Institute in the year 1962. While the name of Haffkine resounds in the heart of every bacteriologist, his life is an interesting adventure in the early days of microbiology. Now, thirty-five years after his death, Haffkine has attracted the attention of many biographers. Two biographies appeared almost simultaneously—one from the U.S.S.R. and the other from the United States. On the hundredth anniversary of the birth of Haffkine, a special postage stamp was issued by the Government of India. This justifies the description aptly chosen by Waksman to end the biography, that Haffkine was "an immortal man of Universe as a whole".

The author has taken elaborate trouble to trace the early life of Haffkine, which was beset with financial difficulties and political as well as religious upheaval against the Jews in Odessa. Perhaps it was then that the spirit of adventure in Haffkine was kindled, as is apparent from the biography.

The leaving of the University of Odessa and his migration to Switzerland and Paris are described as a course of events, which seemed unpurposeful but compelling. It must have been his scientific curiosity, reading and zeal which brought him to the feet of that acknowledged master, Louis Pasteur.

The author has cited with excerpts from "both sides of the story" many comments on the scientific achievements of Haffkine written by his contemporaries. Reading in between the lines, the opposition was mainly motivated for reasons besides those which are normally associated with scientific controversy. His critics never missed the opportunity to attack him right up until his death. Despite all the set-backs that Haffkine encountered in the Malkowal disaster, so well described by Waksman, reason and justice prevailed.

Such a torment to a scientifically inclined missionary in a foreign land did change Haffkine, who returned to India victorious but without his fiery zeal. Not much is known of his later contributions. It was true in Haffkine's life that, if anything is worse than war lost, it is war gained. His later life is described by Waksman as religious. Perhaps Waksman puts his finger on it when he states that he felt his living with Indian people made him change his philosophy of life.

With all this, the author should have paid a tribute to British justice and British recognition of merit despite intrigues that tortured Haffkine. A tribute is also due to Haffkine himself for the metal he was made of to withstand the opposition, insults, injustice and unfair criticism. These need to be inculcated in all scientists and public servants.

The author has taken excerpts from the *British Medical Journal*, the *Lancet* and other medical journals and has made a very correct appraisal of Haffkine's eminence in science. His eminence, however, does not count because of his persecution at any stage of life, though these made Haffkine what he was. He did not seek recognition and he was humane. Persons with such towering personalities, humility and scientific genius need to be highlighted to posterity: Waksman has done a great service to posterity by preparing this biography. No one will be left in doubt after reading this authentic independent adjudgment of Haffkine that he was one of the pioneers in the field of prophylactic bacteriology, to whom the world owes so much.

The book is briefly, but well, illustrated. There appear to be no printing errors. A bibliography of Haffkine and the references cited by Waksman will help medical his-

torians of the future. Science believes either in one world or that it has a world of its own with no barriers. Biography teaches this lesson and here the message is conveyed through Haffkine's life by Waksman's efforts. Haffkine has become immortal in science and his life will continue to inspire microbiologists for a long time.

H. I. Jhala

PLAIN MAN'S GUIDE TO RADIO PROPAGATION

Ionospheric Radio Propagation

By K. Davies. (National Bureau of Standards Monograph 80.) Pp. xiv+470. (Washington: Superintendent of Documents, U.S. Government Printing Office, 1965.) 2.75 dollars.

IONOSPHERIC Radio Propagation is a successor to the National Bureau of Standards Circular 462, published in 1948 under the same title. It is interesting to compare the contents of the two. The new book has more than twice as many pages, but the difference in volume is not great because of the large page size and closely packed layout of the older book.

Not all the new book was written by Dr. Davies, and some sections have been contributed by his colleagues. However, the book is much more of an entity than Circular 462, in which each chapter has separate authorship. The main difference between the books lies in the subjects treated. Some topics such as magneto-ionic theory and an outline of ionospheric physics find an important place in both. The 1948 book fits its title very closely and could be regarded as a handbook for the communication engineer, with substantial chapters devoted to the practical aspects of radio communication. In the 1965 book these topics represent a much smaller proportion of the total. This is largely because they have since been well treated in other publications, to which Dr. Davies refers; but a contributory reason, one suspects, might be that some topics have not progressed sufficiently to warrant extensive coverage. Their place has been taken in the new book by important subjects developed since 1948. These include very high frequency scatter propagation, and low frequency and very low frequency propagation. Another new chapter deals with ionospheric disturbances and their effect on propagation predictions.

With the change of subject-matter has come a change in emphasis. The new book seems to be written not so much for the communications engineer as for a more academically minded readership, the scientific research worker and perhaps the graduate student. So, being personally unfamiliar with some of the topics included, I tried using the book as a tutorial text. In some places the book seemed to fulfil this purpose; in others the exposition did not seem too clear. The book will perhaps be criticized for being too superficial in some places and too detailed in others. In my opinion, books of this type can scarcely hope to escape such criticism, and this one does not seem especially bad in this respect.

The book is provided with 'subject', 'author' and 'place' indexes. The innocent reader might enjoy the index of places; there are naturally experimental stations and scientific institutions (Comfort Cove rubs shoulders with Central Radio Propagation Laboratory), but the entries also include radio stations (GBR, Decca, Loran C); a conference venue (Lindau); and a publishing house (London). The general and the particular are delightfully mixed, as in the consecutive entries, South America, South Ice, South Pole and, again, Scandinavia, Seattle, 75° geographic meridian.

Seriously, this is a useful book. Its clear type, stiff binding and handy page size are a great improvement on the old Circular 462, and at its very modest price it offers excellent value for money.

H. Rishbeth

THE MARINE ATMOSPHERE

Physics of the Marine Atmosphere

By H. U. Roll. (International Geophysics Series, Vol. 7.) Pp. viii+426. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 107*s*. 6*d*.

PHYSICS of the Marine Atmosphere is written by Dr. H. U. Roll, the distinguished German meteorologist and oceanographer. As he claims in the preface, this book sets out to describe marine meteorology as an exact physical science, and so great emphasis is placed on the physical approach to problems of the interchange of properties between the ocean and the atmosphere. The book is probably the first to be published that deals with the subject as a pure science and not as an applied science. There are six chapters which are further sub-divided. The first chapter is an introduction while the second chapter deals with meteorological observations and measurements at sea and contains a review of instruments and methods. The third chapter deals with the composition and properties of the marine atmosphere and includes a discussion of the properties of atmospheric nuclei above the oceans, the chemistry of the marine atmosphere with a sub-section on the still rather contentious subject of carbon dioxide interchange, and also an account of atmospheric radioactivity and electricity.

The backbone of the book is, however, contained in Chapters 4 and 5. Chapter 4 deals with aerodynamic considerations of the interchange of energy between the atmosphere and the sea and contains sections on the geometry of the sea surface and also on the wind profile over the ocean. Chapter 5 gives the thermodynamic approach. Some concluding remarks are given in Chapter 6.

As an oceanographer, I was most interested in Chapter 4. This starts with an account of waves and ripples and gives a brief résumé of recent theoretical work on the subject. I found this part slightly disappointing, however, as the author confines himself almost entirely to theoretical work and to discussing wavelets and ripples, and gives very little attention to the large waves ordinarily found on the sea surface and which must affect the marine atmosphere. It is true that theory cannot as yet fully explain the formation or predict the magnitude of ordinary sea waves and recourse has had to be had to various empirical methods which have not hitherto been in agreement with each other. The problem should not be ignored, however, simply because it has not been solved, especially as recently a much greater degree of agreement has been obtained by various workers about the empirical approach. It is now generally agreed, for example, that the significant height of fully developed waves is given by:

$$H \text{ (in ft.)} = 0{\cdot}018W^2 \text{ (in knots)}$$

where W is the surface wind and full development is reached for fetches less than 400 miles for all wind speeds. This relation should be of interest to both the practical and theoretical marine meteorologist.

The section on the determination of wind profiles over the sea surface is very interesting and it leads on to the evaluation of the wind stress constant over the water surface. There are three traditional methods for determining this parameter. The first obtains it from the wind profile over the first few metres over the surface. The second uses the departure of the mean wind speed from sea-level to the level the geostrophic wind speed is reached from the geostrophic wind speed. The third method is purely oceanographic and involves the measurement of the tilt of the water surface in equilibrium with the wind force. These three methods, however, rarely agree although, to judge from the table shown, there is much better agreement with strong winds. The author criticizes the tilt method generally and in particular a determination in which I had a part, because the effect of the set-up due to wave action is ignored. He suggests that wave set-up

might at least partly explain the apparent increase of stress coefficient with fetch which was found. The effect of wave set-up was certainly not appreciated in 1955 when these results were published, but a recent paper by Longuet-Higgins and Stewart has shown that waves only increase the set-up when their height is limited by breaking, as otherwise the effect is reversed and they reduce the set-up. The recording apparatus in this case was laid in about 2-ft. water depth so the mean wave height should be about 3 ft. for continual breaking, but this value was only very rarely attained during the course of the measurements. The effect of wave set-up is very important nevertheless, and the author rightly points out that tilt experiments should be so designed that this effect is a minimum.

These are all minor points of criticism, and the book is an excellent exposition of the subject and will, I hope, inspire a more widespread study of it. It should certainly be in the library of every oceanographer and every meteorologist.

J. Darbyshire

LIQUID-PHASE OXIDATION OF HYDROCARBONS

The Oxidation of Hydrocarbons in the Liquid Phase

Edited by N. M. Emanuel. Translated from the Russian by K. R. Dobson and B. J. Hazzard. Pp. xv+407. (Oxford, London and New York: Pergamon Press, 1965.) 140*s*.

THE oxidation of hydrocarbons has been the subject of intense investigation for a long time, and particularly so during the past 20–30 years. Oxidation in general is of paramount importance, not only in chemistry, but also in biology and in other related sciences. The oxidation of hydrocarbons is mainly of interest in chemistry, both because of its academic and industrial aspects. In the organic chemical industry oxidation reactions of hydrocarbons play a very important part and great developments are still occurring in this field.

This book deals with certain aspects of the oxidation of hydrocarbons in the liquid phase with gaseous oxygen as oxidant. Even early workers found that the chemistry of these reactions is complex, and it is not surprising, therefore, that progress has been slow. It was established in the early 'thirties that these oxidations proceed by a chain mechanism and show the complex characteristics of reactions of this type. A first real understanding of the mechanism, although confined to the initial stage of the reaction only, was obtained as a result of the work of Criegee and Hock in Germany and of Farmer and Bolland in Britain. This work revealed the central position of hydroperoxides and hydroperoxy radicals in the reaction scheme and led to a mechanism for the first stages of the oxidation of olefines, paraffins, *cyclo*olefines and alkyl-aromatics which has been generally accepted.

However, if oxidation is carried on to conversions greater than a few per cent, complications arise which greatly influence the course of the reaction. This characteristic feature of oxidation reactions is very important because the industrial processes operate in this range of conversion which extends from about 2 to 30 per cent.

During the past ten years or so the Russian workers, whose collective effort is represented in the book under review, have been concerned with this problem and have made important and far-reaching advances in the understanding of the reaction mechanism at the later stages of oxidation. One must be grateful to N. M. Emanuel for having assembled, as editor, his own work and that of Knorre, Maizus, Denisov, Bashkirov, to name only a few, in this book, which has now been translated from the Russian by K. R. Dobson and B. J. Hazzard, and edited by J. D. Hopton.

Apart from this group of papers dealing mainly with the mechanism of the oxidation reaction, the book also contains a collection of papers which are concerned with the technical and more phenomenological, as distinct from the detailed mechanistic, aspects of the liquid-phase oxidation of hydrocarbons.

Discussing first the former group of papers, Emanuel and his colleagues have made good use of the theory of gas-phase oxidation reactions developed by Semenoff. Particularly, the concept of the degenerate chain branching reaction has proved very useful in explaining many features of the liquid-phase oxidation at the larger extent of reaction. In this case degenerate chain branching is due to the decomposition of the hydroperoxides formed as primary products of the reaction. This is the main factor causing the characteristic self-acceleration of the reaction. In addition, other changes in the reaction mechanism occur at this stage. For example, Denisov has shown that the original chain carriers are replaced by others derived from the oxygenated products. The new chain carriers may be less reactive than the original ones and thus slow up chain propagation, resulting in a retardation of the overall reaction.

These complex conditions have been analysed in a quantitative manner, which leads to an explanation of many features of the oxidation reaction at higher conversions. Other characteristics such as the influence of inhibitors of various types also agree with the reaction scheme. These are important achievements well brought out in the group of papers on mechanism. The only criticism I have is a certain obscurity and even incompleteness in the definition and explanation of the symbols used. As a result it is sometimes unnecessarily difficult to follow the mathematical derivations.

As for the group of technological papers, they are concerned with a variety of industrial aspects of hydrocarbon oxidation such as the oxidation of paraffins to fatty acids, *cyclo*hexane to *cyclo*hexanone and *cyclo*hexanol, the oxidation of fuels under storage and the inhibition of this oxidation.

None of the work reported in the latter group of papers is radically new; but it is of great value to have collected in one volume this information, which is otherwise scattered throughout the literature and patent specifications. It should be of particular value to anyone who wishes to orient himself in an unfamiliar field.

This book clearly is to be welcomed and recommended to anybody interested in the oxidation of hydrocarbons in the liquid phase.

The translation is good and readable, and the publishers are to be congratulated for having made the book available to the many who cannot read it in the original language.

H. Steiner

STATISTICS FOR NON-MATHEMATICIANS

Statistical Inference

By Prof. Jerome C. R. Li. Vol. 1: Pp. xix+658. Vol 2: Pp. xiv+575. (Ann Arbor: Edwards Brothers, Inc., 1964.) 10 dollars each volume.

THE first volume of this very large work has the sub-title *A Non-Mathematical Exposition of the Theory of Statistics* and is a revised edition of the author's earlier work *Introduction to Statistical Inference*. The second volume has the sub-title *The Multiple Regression and its Ramifications*, and is a new book. A brief listing of most of the chapter headings will give a fair idea of the contents. Volume 1: descriptive statistics, normal distribution, sample mean, test of hypothesis, χ^{2-}, t^- and F^- distributions, difference between sample means, confidence interval, one-way classification, randomized blocks, linear regression, factorial experiment, analysis of covariance, binomial and multi-nomial populations, transformations, distribution-free methods. Volume 2: matrix algebra, multiple regression, curvilinear regression,

fitting constants, factorial experiments, multiple-factor experiments.

Modern statistics is a mathematical discipline and yet its results are required by people with the very minimum of mathematics, and, in many cases, with a hostility toward the subject. It is therefore one of the hardest tasks a statistics teacher has, to explain his subject to many users of it. Prof. Li's way out of this difficulty (in Volume 1 at least) is to introduce the student to the ideas by means of sampling experiments. Thus, a simple finite population is repeatedly sampled and the behaviour of the sample means discussed. A little further on in the exposition a normal population is similarly sampled and the t-, F- and χ^2- distributions obtained. In this way the student gains familiarity with random variation, can understand how the unusual sometimes happens, and even when, in the more complicated situations, he does not perform the sampling experiments, can appreciate the results in simple terms. A serious disadvantage to this method is that the student does not know which of the conditions of his sampling experiment are essential to the result, and which can be removed. But the author is careful over this point and states the results rather clearly in the form of theorems. With the assumptions on which the results are based stated therein, the student should not fall into the trap that most non-mathematical users of statistics succumb to, and apply the results without criticism in entirely inappropriate situations. The author is similarly very careful over his use of mathematical symbols and introduces them with much explanation.

If the exposition is novel in this respect it is no less novel in its adherence to the normal distribution (or population). Until p. 443 of Volume 1 it is the only population discussed, and no other gets a look-in in the second volume. Even when other distributions (for example, binomial) appear, the methods used involve reduction to normal terms. The extreme example of this is the short (for Prof. Li) chapter of 18 pages on distribution-free methods. He says "the selected [methods] are those which need practically no introduction of new principles". It is not surprising therefore that they "turn out to be the very same methods in disguise".

A second unusual feature is the emphasis placed on multiple regression. This comes relatively early in the exposition and, most remarkably, before the usual constants model for analysis of variance situations. The reason for this is that Prof. Li works through this latter situation in terms of 0, 1, or other appropriate variables and performs a multiple regression with them. Both these novelties have the advantage of giving statistics a unity in that everything stems from the minimum of principles, but I felt that the unity was rather forced.

Without the precision of the mathematical language it is difficult not to make errors. There are quite a few in the 1,233 pages. The most serious is the mis-statement of one of the "only two important theorems given in this text [Volume 2]". This refers to the F-distribution of regression over residual mean-squares, and completely omits to state that it only holds under the null-hypothesis (p. 99). In discussing the limiting form of a histogram (p. 14 of Volume 1) the areas are wrong. The necessity for the two samples to be independent is not mentioned when comparing their variances by the F-statistic (p. 123 of Volume 1), though the point does arise later (p. 137) to be forgotten again on p. 146. No attention is paid to the non-additivity of χ^2 (p. 476). The important point of the transformations being monotone is omitted when discussing the invariance of the median (p. 528).

In addition to errors there are some points which seem unfortunate: the emphasis on significance tests instead of confidence intervals, for example, especially when the former are very firmly of the 'accept-or-reject' type. The unusual notation for regression coefficients (partial and otherwise), which is the opposite of the usual practice, is unnecessary.

These books present an interesting approach to the instruction of non-mathematicians in statistics. If the approach fails, it does so not because the approach is not sound but because it is given in a text which has certain deficiencies, mainly arising out of an emphasis on unity, and is perhaps a little out-of-date. D. V. Lindley

INTERNATIONAL NUTRITION

World Review of Nutrition and Dietetics

Vol. 5. Edited by G. H. Bourne. Pp. x+381. (London: Pitman Medical Publishing Co., Ltd., 1965.) 115*s*.

THE fifth volume of reviews of subjects in the international field of nutrition opens with two essays on nutrition education. The first, by Prof. Beeuwkes of the University of Michigan School of Public Health, gives a good account of the history and the methods of education at all levels in the United States. The second, by Mr. McKenzie and Miss Mumford of Queen Elizabeth College, University of London, presents a critical evaluation of some existing education programmes.

The third paper, from Dr. Todhunter of the University of Alabama, considers some aspects of the history of dietetics and attempts to clarify the differences which may be defined between nutrition and dietetics. It is pertinent to quote the last sentence of the review as a reminder to all who work in the field of nutrition or dietetics. "Food and diet are more than a means of existence, or even of a way to health; they are part of a total way of life and culture of a group and both the nutritionist and dietitian must continue to be cognizant of this fact."

An extensive study of present concepts in diet therapy by Dr. Johnson of the Grace–New Haven Hospital follows Dr. Todhunter's historical review and provides much useful information on normal and modified diets. Adjustments of food intake in quantity and in nutrient content have been seriously investigated in the past forty years, and modern dietary treatment is based on solid scientific investigation rather than the empirical and often fallacious concepts that were accepted in the past.

Some aspects of the metabolism of vitamin A and carotene are reviewed by Dr. Owen of the Hannah Dairy Research Institute, Ayr. Particular attention is directed to the origin and function of liver stores of vitamin A in relation to the composition of blood and milk and the effects of hormones. The functions of retinenes in the vision of various animal phyla are considered. Some recent studies of the relation of vitamin A to enzyme systems concerned with metabolism of sulphate and trace elements are reviewed. Dr. Mahadevan, Dr. Malathi and Dr. Ganguly of the Indian Institute of Science, Bangalore, also review vitamin A in relation to the effect of proteins on absorption of the vitamin. The importance of researches in this field is stressed with reference to the observation that protein malnutrition may precipitate vitamin A deficiency in man.

Dr. Holmes, of the University of Adelaide, makes a useful appraisal of the evidence on which recently recommended protein allowances have been based. Recent recommendations from the United Kingdom, the United States, the Food and Agriculture Organization and Australia are compared with critical reference to discrepancies.

An extensive documented review of bone pathology in experimental malnutrition is given by Mr. Stewart of the Human Nutrition Research Unit, National Institute for Medical Research, London. The final paper of the volume is concerned with the fate of selenium in animals. It comes from Dr. Ganther of the University of Wisconsin.

The nine reviews add up to another valuable collection of information of general interest. A. M. Copping

CHARACTER RECOGNITION BY HOLOGRAPHY

By Prof. D. GABOR, F.R.S.
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WAVE-FRONT reconstruction or holography, on which the first report[1] was published in *Nature* seventeen years ago, had a powerful renaissance in the past years. E. N. Leith and J. Upatnieks[2], G. W. Stroke[3] and others have greatly improved the original method, and showed that it was possible to reconstruct complicated two- and three-dimensional objects, with half-tones, in previously unattainable perfection. The revival of holography owed much of its impulse to the invention of the laser, which made it possible to produce holograms with interferences of the order of 10,000, and thus to make full use of the information capacity of fine-grain photographic plates.

I wish to show that it has now become possible to harness holography for the solution of one of the most urgent problems of computers and other data-processing devices; the recognition of characters with many variants.

Wave-front reconstruction contains a principle which has not yet been fully exploited. Expressed in a general form: two coherent waves are made to fall simultaneously on a photographic plate, one coming from an object A, the other from an object B. The photograph links these together in such a way that if the hologram is illuminated by A alone, B will appear too, and vice versa. So far this principle has been applied in the form that A was the object of interest and B a light source, usually a simple one, and in the reconstruction the hologram was illuminated by B. I now propose to turn this around. Let A be a character, such as a printed or hand-written letter or numeral, which can be read by human beings but not by a machine, and let B be a combination of point-sources, forming a code-word which can be read by a machine. Produce the hologram by combining A and B. When A, or a character sufficiently close to it is presented to the hologram, with the original illumination, the code-word B will flash out. This means that the hologram can act as a translator, or coding device.

The interest of this principle is in the enormous recognition capacity which can be stored in a single hologram, and which one might not perhaps suspect at first sight. I wish to show that with N characters to be discriminated, each with M variants, the product $M.N$ can be made of the order of a thousand or even more.

Fig. 1 shows the optics for producing the master hologram, and for using it in the read-out. The recording medium is assumed to be a transparency, such as a microfilm; but reflecting media can also be used. The hologram is built up by repeated exposures in what may be called 'layers'. These are not, of course, physically separated in the emulsion. Each layer corresponds to one of the N characters to be discriminated, with all its M variants, and is marked with one code-word. The layer contains the part-holograms, to be called 'engrams' of the variants side by side, with little overlap. Each engram is produced with one direction of illumination, and as the photographic plate is arranged in the rear focal plane of a lens viewing the character, it is a 'Fourier-hologram'. This has the advantage that the hologram is translation-invariant, that is to say, independent of the position of the character so long as this appears alone in the window. An engram need not occupy much more area in a photographic plate than would be needed for a good record of the corresponding character, but as a cautious example we will assume 120 engrams, each with a diameter of about 5 mm, on a photographic plate of 50 mm × 50 mm. This is sufficient to record without overlap 30 variants, each in four or six 'identical' engrams. Fig. 1 shows how this is achieved.

The light of a laser issues from a point L, and a beam splitter consisting of a spherical mirror and a semi-reflecting mirror produces of this two images L' and L''. The first of these serves the illuminator; the second, in the centre hole of the illuminator plate, serves the code plate. The illuminator plate, backed by a field lens, consists of a plastic plate embossed with, say, 120 lenticules, and is black outside the lenticules. These produce 120 point sources which illuminate the window containing the character through a lens 1, which removes the illuminator points into star space. The point sources correspond one-by-one to the engrams. There is a certain advantage in randomizing them slightly. Four identical engrams are taken at a time of any one variant. These are spaced out, as far as possible, to increase the resolving power of the hologram. They are selected with a mask, and a different mask with four holes is used for every variant.

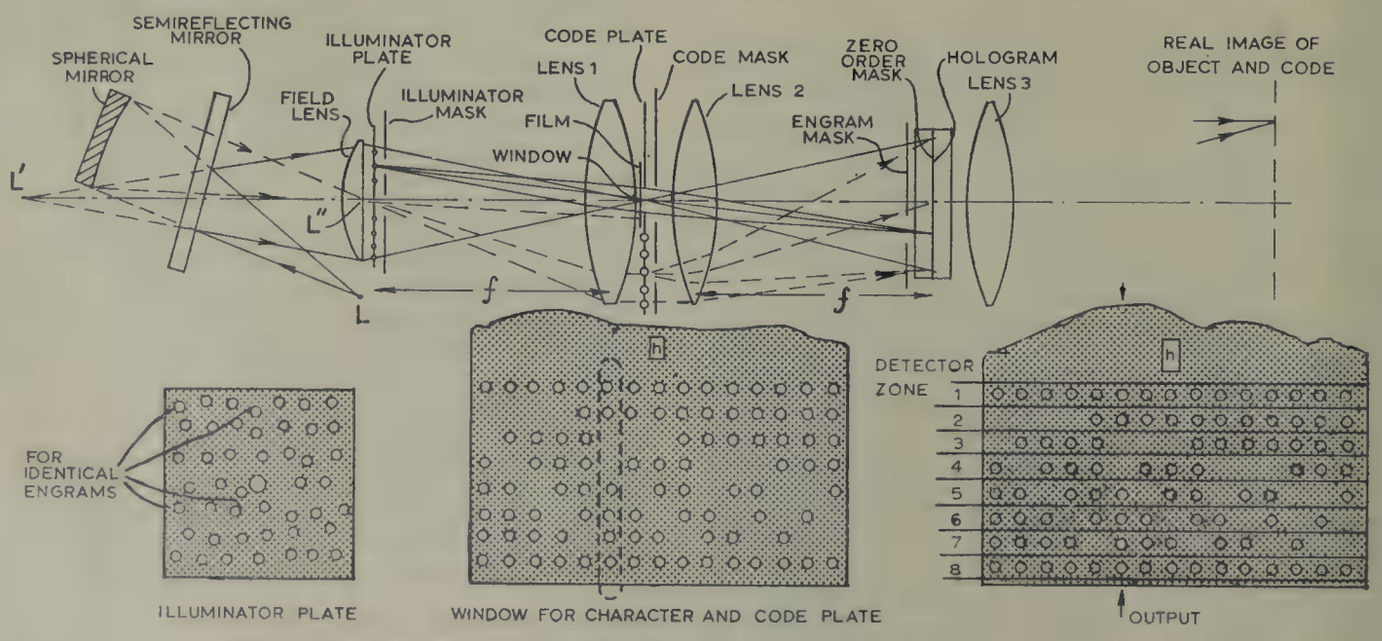

Fig. 1. Apparatus for producing a coding hologram and using it for the read-out

The point source L'' in the centre of the illuminator plate illuminates the code-plate, through the same lens 1, which serves in this area as a field lens. The code-plate, like the illuminator, is an embossed plastic plate, which contains the code-word in the form of groups of luminous points, arranged in one or several arrays. It is advantageous to use self-checking codes, in which every word has the same number of code-points. In the example there are six positions, of which two remain dark and four light up. This code has $6.5/1.2 = 15$ words. Two more positions have been added. These do not contribute to the discrimination of characters, but improve the signal-to-noise ratio, as eight points have to light up for every valid character.

In the making of the master hologram all engrams in one layer are marked, that is to say, exposed simultaneously, with one distinctive code-word, which is selected by a mask. But as each code-word illuminates the whole area of the hologram, a further mask must be used near the plane of the photographic plate, which cuts out the light except in the area of the engrams which are made at any one time. This makes it possible to observe the rule of optimum illumination, which postulates about equal light sums on any engram from the character and from its code-word.

Black-on-white letters are less suitable for discrimination than their negatives, because they have too much in common; all their white area. But this disadvantage can be eliminated by a further mask, in the plane of the hologram, which cuts out all undiffracted light. By Babinet's principle this turns a character into its negative. Such a mask can be easily made by exposing a photographic plate through a clear window simultaneously to all illuminator points.

After $M.N$ successive exposures of the photographic plate, which add up to a convenient medium density, the master hologram is made by processing and printing it, preferably with an overall gamma of 2, and the print is put back in the original position. In the reading all the point-sources of the illuminator are used, while the whole code-plate is covered up. A lens 3 is used for observation, which produces a real image of the code-plate. If now the recording medium is dragged across the window, whenever a character or a variant appears in it, its code-word will flash up. It is advantageous to arrange in the image plane a mask, which is a replica of the code-plate, with very fine holes, so as to exclude all but the signalling light. This mask, too, can be made photographically.

A method of reading the code-words is to sum up all the light which appears in one zone, corresponding to one position in the code, and guide it to a separate photoelectric detector. Each detector is fitted with a level discriminator, so as to reject spurious signals below a certain level. This method is simple; but it has only moderate discriminating power, because if the characters are not clearly distinct, some light might show up in the same zone in the code-words of other characters. One can reduce this by making the code-words of characters which are not clearly distinct as different as possible. But the maximum of discrimination is achieved by a somewhat more complicated apparatus. In this the image of the code-plate is projected on the screen of an image camera. The code-words flash up at intervals corresponding to the time allotted to each letter, during 10–30 per cent of this period. In the time between flashes all code positions are scanned word by word, and points above a certain level of intensity are transferred to a memory organ, such as a core store. But unless the full number of points appear in a word, the record is erased. If the full number is counted, the code-word is transferred to the computer.

The great discriminating power of the holographic method stems from its high angular resolution. Assume, for example, $N = 35$, $M = 30$, $M.N = 1050$. The group of four engrams corresponding to the character presented to the reader receives 1/30 of the light, and can diffract about 1/35 of it, altogether about 10^{-3} of the total. (Not counting, of course, in black-on-white records, the undiffracted light which goes into the zero order.) Of the diffracted light, under the proper conditions, that is to say, when the engrams were taken with about equal light sums from the letter and from the code-word, one-quarter will go into the reconstruction of the code-word. One half appears in the object, another quarter goes into the 'twin' image of the code-word, which, however, is washed out by intermodulation with the character, and is useless for recognition. But the useful quarter is concentrated in extremely small solid angles. For example, if four or six identical engrams are spaced out by about 25 mm, the solid angle in which the major part of the light corresponding to a code-point is concentrated will be of the order 10^{-8}. Let the light of, say, 10^{-4} of the total be distributed among ten code-points, this means that 10^{-5} of the light appears in one code-point, in a solid angle which is perhaps 10^{-6} of the solid angle covered by the whole code; a concentration of the order ten. Moreover, this estimate is somewhat pessimistic, because it takes no account of the confirmation of the character by the engrams of slightly different variants in the same layer.

In conclusion, there is good reason to believe that a single hologram may discriminate between all the numerals and the letters of the alphabet, each with 30 variants.

[1] Gabor, D., *Nature*, **161**, 777 (1948); *Proc. Roy. Soc.*, A, **197**, 475 (1949); *Proc. Phys. Soc.*, B, **64**, 244 (1951).

[2] Leith, E. N., and Upatnieks, J., *J. Opt. Soc. Amer.*, **53**, 1377 (1963); **54**, 1295 (1964); **55**, 569 (1965).

[3] Stroke, G. W., *Optics of Coherent and Non-coherent Electromagnetic Radiations, Univ. Michigan* (1965), with Falconer, D. G., *Physics Letters*, **13**, 306 (1964); **15**, 283 (1965).

POLONIUM-210 IN MAN

By Dr. C. R. HILL

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IT is well known that the atmosphere contains appreciable quantities of radon as a result of emanation from the materials of the Earth's crust. Until recently, however, little interest has been taken in the fate of the long-lived radionuclides produced in the atmosphere by radon decay. Radium D (lead-210) has a half-life of twenty-two years, much longer than the twenty–thirty day mean residence time for aerosols in the troposphere, and it is therefore returned to the surface of the Earth as a natural component of fall-out. During residence in the atmosphere, sufficient time elapses for the growth of most of the equilibrium amount of the five-day β-emitter radium E (bismuth-210) and some of the 138-day α-emitter radium F (polonium-210), and these therefore also take part in the fall-out process.

In addition to this natural process of production, polonium-210 may be released into the atmosphere by a number of artificial means. Considerable quantities are thought to have been released by the Windscale accident in 1957 (ref. 1). It is being used as a source of thermal power in Earth satellites and there has recently been interest in the possibility that a certain amount may have been

produced by activation of stable lead or bismuth in nuclear weapon explosions. Except, possibly, in the stratosphere the natural source of the nuclide is predominant at the present time, but this may not always remain the case.

Burton and Stewart, in 1956, measured the concentration of radium *D*, *E* and *F* in ground-level air and in rain-water at Harwell[2] and their figures show an annual deposition rate for radium *D* of about 1·7 mc./km² (ref. 2), which is nearly half the corresponding mean rate for strontium-90 recorded at the same site during the past five years[3]. The concentration of radium *D* in ground-level air appears to be dependent on latitude, being highest in temperate latitudes and also being generally higher in the northern than in the southern hemisphere[4], possibly as a result of the difference in exposed land masses.

Dispersion as an atmospheric aerosol is well known to be an effective means by which many trace materials may gain entry to the biosphere. Lead, bismuth and polonium may all be absorbed by human beings as a result of inhalation and also to an appreciable extent by uptake through the gut[5]: it is therefore of interest to know to what extent human beings may be exposed to these nuclides in the environment and whether, in fact, they are normally present in human tissues.

From knowledge of the energy and linear energy transfer of the radiation emitted by the three nuclides radium *D*, *E* and *F*, it appears that the respective biologically effective doses produced per disintegration will be in the approximate ratios 1 : 10 : 1,000 (assuming an RBE of 10 for α-particles). It is radium *F* (polonium-210) that is of primary interest, therefore, in the present context, although it must be borne in mind that its actual presence and pattern of distribution in the human body may be determined very largely by the behaviour of one or more of its radioactive precursors.

Human and animal foodstuffs. Polonium-210 has been observed to occur on exposed foliage, and particularly on grass, in amounts that are directly related to local rainfall[6]. In high rainfall districts in England and Wales concentrations above 10,000 pc./kg of dry grass are not uncommon, with the curious consequence that a load of hay can become legally classifiable as a 'radioactive substance' purely on account of its natural radioactivity. Concentrations of the order of 5,000 pc./kg have also been found to be common in slow-growing plants such as lichens, both in the United Kingdom and also in Lapland and the Canadian Arctic, where they form an important part of the winter diet of reindeer and caribou.

Grazing animals are found to take up polonium-210 in appreciable amounts, and concentrations greater than 1,000 pc./kg have been found in some animal tissues both in the Arctic and in high rainfall areas of the United Kingdom. Biological concentration of polonium-210 also occurs in some marine organisms and in particular in some varieties of plankton[7], certain shellfish and crab. Evidently, therefore, there is a considerable variety of ways in which the nuclide may find its way into human and animal foodstuffs, and the amounts that we have measured in a number of these*, together with observed values of the lead-210 : polonium-210 activity ratio in certain cases, are shown in Table 1.

Table 1. Polonium-210 Content of Various Human and Animal Foods

Materia	No. samples	^{210}Po spec. activ. (pc./kg)	^{210}Pb : ^{210}Po activ. ratio
Grass (dried) U.K.	24	400–16,000	1–5
Dry lichen (*Caloplaca elegans*) U.K.	2	7,800; 10,000	1
Dry lichen (*Cladonia alpestris*) Lapland	3	6,600–8,100	1
Dry lichen (*Cladonia alpestris*) Canada	1	3,500	1
Edible green vegetables (U.K.)	5	6–90	1–3
Carrots and potatoes (U.K.)	2	1	—
Breads and cereals (U.K.)	4	1–7	—
Dried milk powder (U.K.)	3	2–6	—
Beef and lamb muscle (U.K.)	2	3 ; 3	—
Beef and lamb liver (U.K.)	3	4–100	0·7
Beef and lamb kidney (U.K.)	3	48–270	0·05–1
Lamb kidney (N. Wales)	6	90–1,800	0·2
Reindeer (Lapland) summer killed			
Muscle	6	15–50	—
Liver	5	350–750	—
Kidney	4	110–490	—
Bone	5	520–3,500	—
Reindeer (Canada, N.W. Territory) winter killed			
Muscle	2	200 : 210	—
Liver	2	2,400 : 5,600	—
Kidney	2	4,200 ; 2,300	—
Spleen	1	980	—
Camel bone (Jordan)	2	1,100 ; 1,500	—
Deer bone (Scotland)	1	1,300	—
Cockles (U.K., E. and W. coasts)	3	400–900	0·1–0·2
Crab (U.K., S. coast)	2	1,300 ; 1,400	—
Plankton (S. Pacific)	1	2,000	—

The data of Table 1 are somewhat fragmentary and are characterized by wide variations in the concentrations of the nuclides, but it may be concluded that the daily intake of both polonium-210 and lead-210 in the food comprising an average mixed western diet will probably be in the range 1–10 pc. This estimate is supported by measurements we have made of the daily faecal excretion of lead-210 by a thirty-year-old male subject consuming an ordinary 'English' diet. For six separate samples collected at intervals over five months the values were between 1·7 and 6·4 with an average of 3·2 pc./day. From similar measurements in the United States, Holtzman has obtained a value of 1·8 pc./day[8].

Evidently a considerably higher figure might apply, for example, to a connoisseur of sea food, while an Eskimo on a high meat diet based on winter-killed reindeer might ingest of the order of 100 pc./day of each nuclide. It is to be noted that the polonium-210 and lead-210 contents of drinking-waters (other than untreated rain-water) are only of the order 0·01 and 0·05 pc./l. respectively[9] and so would probably be insignificant in comparison with food as a source of ingestion of the nuclides.

Tobacco and cigarette smoke. It has been pointed out by Radford and Hunt that appreciable concentrations of polonium-210 occur in tobacco and that, as might be expected from the known volatility of polonium, a considerable fraction of the activity in the tobacco may be transferred to the inhaled smoke[10].

I have measured polonium-210 concentrations in a number of cigarette tobaccos of different origin, with the results shown in Table 2. These figures do not indicate any striking differences as between the different major tobacco-producing areas and, in particular, do not support the suggestion recently made by Marsden[11], based apparently on total α-activity measurements only, that differences in the polonium-210 content of tobaccos might be related to geographical variations in the incidence of lung cancer.

We have also investigated the fate of the polonium-210 which is present in a cigarette when it is smoked in a standard smoking procedure†. Using a popular brand of cigarette which was found to contain 0·49 ± 0·07 pc. polonium-210 per cigarette, the fate of the original activity in three experiments was as shown in Table 3, where the previous results of Radford and Hunt are summarized for comparison. It will be seen that the main conclusion from the earlier work, that an appreciable fraction of the polonium appears in the main-stream

* Apart from a limited number of α-spectroscopy measurements, the polonium-210 determinations reported in this article have been carried out by the method described by Black (ref. 27), Osborne (ref. 12) and others. Samples are wet ashed using nitric and perchloric acids and polonium is then deposited electrochemically on to silver from a solution in 0·5 N hydrochloric acid, and afterwards α-counted. Yield is checked using duplicate samples spiked with a standard ^{210}Pb–^{210}Po solution and lead-210 can be estimated by replating polonium-210 from the solution after an interval of several months.

† Smoking was carried out under the following conditions, using a bellows type smoking machine: initial cigarette length 70 mm, weight 1.09 g; butt length 23 mm; puff volume 35 ml., duration 2·0 sec, repeated once a minute to total 10 puffs per cigarette; horizontal smoking position. In experiment No. 3, filter tips were fitted to the cigarette, using adhesive tape. Mainstream smoke (that is, the fraction normally drawn into the mouth) was drawn directly into the collecting device, which was either a Cambridge filter (expts. Nos. 2 and 3) or two wash bottles containing 5 per cent hydrochloric acid in ethanol and immersed in solid carbon dioxide (expt. No. 1). Side-stream smoke was led through a collecting chimney and collected either in two similar wash bottles in series followed by two Cambridge filters in parallel (expt. No. 1) or in the two filters only (expts. Nos. 2 and 3). Following collection, smoke was recovered from the filters by gently flowing hydrochloric acid–ethanol mixture in the reverse direction through the filters, still in their holders. Polonium-210 estimation was carried out as described in the previous footnote.

Table 2. Polonium-210 Content of Various Cigarette Tobaccos

Origin of sample	No. of samples	^{210}Po activity (pc./kg) Max	Min	Mean
United States	8	650	390	510
Cent. and S. America	6	1,360	290	670
Rhodesia	3	700	600	650
Australia	2	660	610	640
India and Pakistan	2	570	250	410
Indonesia	1	—	—	230
Turkey and Greece	3	280	210	240

Table 3. Fate of Polonium-210 in Smoking of Cigarettes
(Percentages of total initially present)

Component	Present measurements Expt. 1 (no tips)	Expt. 2 (no tips)	Expt. 3 (filter tips)	Radford and Hunt[10] (No tips)	(Filter tips)
Main stream	12	10	6·5	23; 25	22; 18
Tips	—	—	3·5	—	—
Side stream	32	28	30	21; 29	27; 24
Butts	35	34	37	30; 25	24; 38
Ash	16	18	19	7; 11	9; 8
Total recovery	95	90	96	81; 90	82; 88

smoke, is confirmed, although the actual fractions found in the present experiments are somewhat lower, possibly as a result of differences in smoking conditions.

In a separate experiment, main-stream smoke from cigarettes from the same batch was weighed after collection on Cambridge filters and found, in two separate measurements, to amount to 0·032 g per cigarette as collected or 0·021 g after drying at 100° C. Taking the polonium-210 content of main-stream smoke as being about 0·05 pc. per cigarette (from the foregoing measurements) it is evident that the specific polonium-210 α-radioactivity in the condensed smoke is of the order of 2 pc./g. The radiation dose rate at the surface of a thick (that is, > 100μ) slab of such material would be 0·1 rad (or 1 rem) per year and less for thinner slabs. This is of interest in considering the possible occurrence of high local dose rates in the human lung as a result of accumulation of smoke particles, since the foregoing dose rate is in marked contrast with the values of from several hundred to more than 1,000 rems in twenty-five years suggested by Radford and Hunt[10]. For such high dose rates to occur it would seem to be necessary to postulate a mechanism for selective clearance from the lung of the bulk constituents of the condensed smoke relative to that of polonium-210. Such a mechanism would not only have to show a high degree of selectivity but also, on the evidence given later for retention time in the lung for the polonium-210, would need to operate rather rapidly.

Normal human tissues. A number of measurements have been reported of the concentrations of polonium-210 and lead-210 in normal human tissues, although, apart from those by Osborne[12] and Stahlhofen[13], most have been concerned only with bone and teeth[8,14,15]. In view of the results presented here, I have made two further sets of measurements on human tissues. In the first place I have examined the polonium-210 levels (equivalent to lead-210, as the samples were several years old at the time of measurement) in bones of ten different Canadian Eskimos. Secondly, I have made comparative measurements of polonium-210 on soft tissues of two groups of individuals known to be non-smokers and regular cigarette smokers respectively, all of whom died sufficiently suddenly that their smoking and other habits would not have been upset seriously for more than a few hours before death. Measurements were made without subdivision of the tissues except in the case of the right lung, where the bronchial tree and samples of peripheal alveolar tissue were dissected out and measured separately. In three cases the secondary bronchial bifurcations were dissected out (to include about 0·5 cm of bronchus above and below the bifurcation) and measured separately. The results of measurements on the Eskimos and on the effect of smoking are presented in Tables 4 and 5 respectively.

The measurements on the Eskimos can be compared with values found by other investigators for bone of 'normal' European and North American subjects (shown in the same Table). From this it will be seen that the Eskimo values range from 'normal' upwards by a factor of twenty or more, and it is interesting that the high values occur in subjects who had been permanently resident in the Arctic while several of the others had been living in southern Canada for some time prior to removal of the bone (either at autopsy or in the course of thoracoplasty). The high values found here seem to be consistent with my observations of high levels in reindeer meat and the known high meat diet of Eskimos.

(*Note added in proof.* Recent measurements on fresh placental tissue samples derived from nine northern Canadian residents whose diet included reindeer or caribou meat have shown polonium-210 concentrations

Table 4. Lead-210 Levels in Bones of Canadian Eskimos
(Measured as polonium-210 six years after collection)

Case No.	Origin and age of subject (where known)		^{210}Pb activity (pc./kg wet bone)
1	Igloolik, N.W. Territory	42	710
2	—	—	380
3	Carberry, Man.	67	8
4	Winnipeg, Man.	80	13
5	Edmonton, Alta.	44	27
6	—	—	12
7	Pakatawagan, Man.	21	37
8	Nelson House, Man.	25	42
9	Nelson House, Man.	17	40
10	—	—	100
"Normal" (United Kingdom and United States, refs. 8, 12, 14 and 15)			15–37

Table 5. Polonium-210 Tissue Levels in Smokers and Non-smokers

Age and sex of subject	^{210}Po Activity (pc./kg tissue) Liver	Bronch. tree	Alveolae	Mean lung (approx.)	Kidney	Gonad	Activity ratios Lung/liver	Kidney/liver	Bronch. tree/ alveolae
Cigarette smokers									
23 M	7·7	6·5	7·2	6·8	13·8	4·0	0·88	1·8	0·9
22 M	22	6·0	10·1	8·0	21	3·6	0·36	0·95	0·6
62 M	27	10·2	15·6	12·9	24·5	—	0·48	0·90	0·65
56 M	23	6·6	6·6	6·6	22·7	4·1	0·29	1·0	1·0
Mean	20	7·3	9·9	8·6	20·5	3·9	0·43	1·02	0·74
Non-smokers									
68 F	7·6	2·0	1·2	1·6	5·7	—	0·21	0·75	1·6
66 F	12·5	1·2	2·0	1·6	—	—	0·13	—	0·6
54 F	19·7	1·9	1·9	1·9	11·4	1·7	0·10	0·58	1·0
63 F	13·2	1·6	1·3	1·4	7·9	—	0·11	0·60	1·2
17 M	12·8	4·0	3·8	3·9	10·5	4·0	0·30	0·82	1·0
57 F	22·6	7·7	10·2	9·0	38·5	—	0·40	1·7	0·75
Mean:	14·8	3·1	3·4	3·2	15	2·8	0·25	1·07	0·75
Measured ^{210}Pb/^{210}Po ratios									
Max	1·0	—	0·7	—	0·6	—			
Min	0·1	—	0·5	—	0·14	—			
Mean	0·47	—	0·6	—	0·31	—			

twelve times greater, on average, than those found in nine other subjects from the same area.)

From the measurements on smokers and non-smokers (Table 5) the following points emerge:

(1) In both smokers and non-smokers the ratio between gross specific polonium-210 activity in the bronchial tree and that in alveolar tissue is close to unity for any individual.

(2) In liver and kidney the levels of polonium-210 in smokers are little, if at all, greater than those in non-smokers, whereas in lung tissue a difference with a factor of between 2 and 3 seems to occur. This is perhaps best seen in comparison of the activity ratio 'lung/liver' for the two groups. The corresponding ratio 'kidney/liver' may here be regarded as a control parameter indicating variations occurring between two tissues neither of which is directly concerned in the exposure mechanism of interest (inhalation).

(3) In my samples the polonium-210 content of the average smoker's lung exceeds that of the average non-smoker by 5·4 pc. (assuming 1 kg for the total weight of the lungs), which is the approximate content of the nuclide in the main-stream smoke of a hundred cigarettes. Assuming a consumption of twenty cigarettes per day and retention by the lung of half the main-stream smoke, these results suggest a retention time for the polonium-210 in the lung of the order of ten days.

(4) For the three cases of smokers where bronchial bifurcations were dissected out and measured separately, the polonium-210 in each sample (of weight between 5 and 15 g and each consisting of several bifurcations) did not in any case exceed the amount to be expected, from the average value measured for the whole bronchial tree, by more than 0·05 pc.

In view of the possibility suggested by Radford[10] that even such a small quantity of activity might lead to high dose rates if it were concentrated into a small area, I have examined autoradiographically some vacuum-dried samples of epithelium taken from bronchial bifurcations in smokers' lungs. In the specimens examined so far I have not found statistically significant concentrations of α-activity greater than 0·01 pc./cm^2, and an appreciable part even of this activity is probably attributable to background from the glass plates used in the initial experiments.

My failure to find appreciable excess polonium-210 activity in bronchial bifurcations of cigarette smokers, either when measured as gross tissue concentrations or when examined autoradiographically as surface activity, is in contrast with Radford's observation of hot spot activity[10] and also with his later observations of polonium-210 activities up to 1·25 pc./g in bronchial epithelium[16].

Subjects with high skeletal burdens of radium-226. The decay of radium-226, present in the human skeleton as a result of either natural or abnormal exposure, leads eventually to the formation within the body of a certain amount of lead-210 and its descendants. It will be shown later than in the normal subject this source is of little importance, but in subjects with high radium burdens it can constitute the main source of these nuclides and is of interest therefore as providing an opportunity for an investigation of the effect of differences in the route of their administration to the human body.

I have recently had occasion to examine *post mortem* a subject who had been employed as a radium-dial painter until six weeks before her death, which resulted from acute myeloid leukaemia. Her body-burden of radium-226 proved to be 0·02 μc.; well below the maximum permissible level, but about one thousand times above normal. The levels of polonium-210 and lead-210 measured in various organs at the time of death are shown in Table 6, both as absolute amounts and as ratios to the corresponding average levels that we have measured in normal individuals. Several interesting points emerge from these figures:

(1) In bone and liver the presence of polonium-210 appears to be due to the quantitative retention of the products of decay of lead-210 in the tissue, since the two nuclides occur in equilibrium. In kidney and ovaries, however, the polonium-210 is significantly in excess of equilibrium and is presumably present largely as a result of active uptake of polonium from the blood. The discrimination between the renal cortex and medulla is in agreement with animal data and with a single previously published case of a human subject[17].

(2) The exposed/normal ratio for polonium-210 in a tissue is much higher for bone than for most soft tissues. This presumably reflects the known fact that an appreciable fraction of the radon produced in bone by decay of radium-226 does not escape but decays there with the eventual deposition of lead-210 and polonium-210. (The high absolute and relative levels of polonium-210 in hair may be due to superficial contamination by radon decay-products in the atmosphere of the luminizing factory.)

(3) The actual distribution of polonium-210 among the soft tissues does not seem to be strongly dependent on the origin of the polonium. It is of particular interest that this observation applies also to the lung: this could be a fortuitous result of the inhalation of high concentrations of radon and daughters, but otherwise it suggests that much of the normal polonium-210 content of the lung originates internally rather than as atmospheric material that has not been cleared physiologically.

(4) In relation to the radium-226 content of the skeleton, the ^{210}Pb–^{210}Po levels in the soft tissues of the exposed subject are seen to be considerably less, by a factor of about 70, than those in normal individuals. This is in agreement with Holtzman's prediction[8] that the normal individual acquires only a very small fraction of his polonium-210 burden (at least in soft tissues) as a result of decay of skeletal radium-226.

Measurements have also been made of polonium-210 in the tissues of a number of subjects having radium-226 burdens that had been acquired some twenty–forty years before their death. The results, which are given in Table 7, show a level of polonium-210 in soft tissue, for a given radium-226 burden, that is considerably less than that in the more recently exposed case. This situation is consistent with the older radium deposits being less subject to loss of radon to the blood, but it could also be explained on the basis of high inhalation of radon and daughters by the more recently exposed subject.

If the commonly used value of 0·67 is assumed for the fraction of radon-222 produced in bone that escapes to the blood, the data of Table 7 may be used to calculate the rate of uptake of radon-222 into the blood that would by itself maintain the normal concentration (10 pc./kg) of polonium-210 in the liver. A value of approximately 0·5 pc./min is found in this way.

Table 6. Polonium-210 and Lead-210 Levels in Tissues of a Radium-dial Painter

Tissue	^{210}Po activity (pc./kg)	^{210}Pb : ^{210}Po activity ratio	^{210}Po ratio exposed : normal[12]
Liver	190	1·0	19
Lung	50	—	15
Kidney cortex	360	0·13	40
Kidney medulla	200	0·10	40
Spleen	34	2·5	11
Ovaries	60	0·25	9
Thyroid	11	—	—
Pancreas	8	—	6
Adrenals	70	—	—
Hair	25,000	1·0	280
Bone	1,500	1·0	88
Bone—^{226}Ra	4,000	—	1,000 (^{226}Ra)

Table 7. Radium-226 in Bone and Polonium-210 in Liver for Individuals with Long-established Radium Burdens

Approx age of ^{226}Ra burden (years)	^{226}Ra in bone at death (pc./kg wet)	^{210}Po in liver at death (pc./kg wet)	Ratio: bone ^{226}Ra/liver ^{210}Po
20	9,000,000	11,000	800
40	2,300,000	2,000	1,100
35	2,000,000	4,500	450
30	80,000	70 (normal)	(1,000)
20	40,000	110 (normal)	(400)
40	30,000	80 (normal)	(300)

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Isolated proteins. One of the biological properties of polonium that is often overlooked, although it has been known for some forty years[18], is that it is a 'seeker' of soft tissues rather than bone, and it is in fact unique in this way among the α-emitting heavy elements. (The common occurrence of polonium-210 in bone is almost certainly not a result of its own chemical nature but rather of its descent from the bone seeker lead-210.) The reason for this behaviour is not fully understood, although it is known that polonium may be associated with protein, particularly in the blood[19,20].

In this connexion it seemed of interest to determine whether the polonium-210 that has been shown to occur in grazing animals may be present in nominally pure crystalline proteins extracted from their tissues. The results of several such measurements on commercially prepared samples are shown in Table 8 together, for one of the insulin samples, with corresponding measurements both on the pancreas from which the sample was derived and on a multiply recrystallized fraction of the commercial grade of protein.

Table 8. Polonium-210 Contents of some Pure Proteins

Protein	Supplier	^{210}Po activity (pc./kg)
Albumin (crystalline bovine)	B.D.H.	23
Haemoglobin	B.D.H.	96
Gelatine (powder)	B.D.H.	40
Fibrinogen (bovine)	B.D.H.	120
Insulin (crystalline bovine)	B.D.H.	610
Insulin (crystalline bovine)	Boots	1,870
Insulin (crystalline bovine)	Burroughs Wellcome	660
Insulin (crystalline bovine) (multiply recrystallized)	Burroughs Wellcome	370
Raw bovine pancreas (dried)	Burroughs Wellcome	25

The difference in activities between the insulins and the other bovine proteins might simply reflect a difference in origin (the three commercial insulins, although from different manufacturers, were all derived from a common source of supply of pancreas), but it is of interest that, of the various proteins examined, insulin has much the highest content of —SH groups. The reduction in specific activity on multiple recrystallization appears to rule out a very close association between the polonium and the protein, but the possibility is nevertheless suggested that some association, in which —SH groups are involved, may exist. Such a situation would evidently have important implications for the microdosimetry of the polonium isotopes.

Discussion

The selection of data presented in this article has been dictated to a considerable extent by the availability of post-mortem and other specimens and is therefore far from comprehensive. Nevertheless, from these results and others previously reported in the literature, at least the outlines of a consistent picture can be obtained of the part played by the sub-series radium *D–E–F* in man's radioactive environment.

The correspondence, both for grazing animals and for man, between the levels of polonium-210 in the food of a community and in the tissues of its members clearly indicates that ingestion in food is an important route for acquisition of body-burdens of the nuclide. The conclusion that this is normally the principal source of such body-burdens seems to follow from Holtzman's analysis of other possibilities[8], including decay of skeletal radium-226 and inhalation of radon-222, radium *A*, *B* and *C* and radium *D*, *E* and *F*, in which he showed that only the last-mentioned of these is of comparable importance with food, and that only if one assumes an atmospheric concentration of the nuclides several times higher than has been measured by most observers[2,4].

The variations, by an order of magnitude or more, between polonium-210 body-burdens in 'normal' United Kingdom and United States residents and in some Canadian Eskimos, are probably due to the latter having a high dietary content of meat derived from animals grazing on poor, slowly growing pasture. Apart from in the Arctic, such conditions might also be expected among the herdsman populations of arid regions such as those in Arabia and East Africa, and it is of interest here that we have found high levels of polonium-210 in camels.

Cigarette smoking does not seem to lead to abnormal levels of polonium-210 in tissues other than lung, and the levels here, examined either as gross tissue concentrations or, so far as I have been able to discover, as localized concentrations, are not such as would lead to abnormally high radiation dose rates. However, the possibility of being able to observe localized concentrations of smoke particles in the bronchial epithelium by virtue of their association with a radionuclide is of considerable interest, regardless of considerations of radiation dose to the tissue, and would justify further experimental effort in that direction.

The question of the part played by physiological uptake of lead-210 in producing tissue concentrations of polonium-210 is still not completely settled[21,22]. Evidence from work with experimental animals, as summarized by the International Commission on Radiological Protection[5], indicates that the steady condition reached following prolonged chronic ingestion of an equilibrium mixture of lead-210, bismuth-210 and polonium-210 would be one in which lead-210 was considerably in excess of polonium-210; the predicted ^{210}Pb : ^{210}Po ratios for bone, liver and kidney being 400, 32 and 25, respectively. Values for this ratio between these figures and unity would still be consistent with the data from the International Commission on Radiological Protection if a fraction of the decay products of lead-210 was retained in the tissue where it had been formed, whereas values less than unity could only be explained by an active uptake of polonium-210 into the tissues (or, possibly, by redistribution of polonium-210 between different tissues[22]). In fact, I have repeatedly found values for this ratio considerably less than unity in a number of soft tissues and consistently close to unity for bone. It appears, therefore, that, under the conditions we are studying, there may be a true uptake of polonium-210 into certain tissues independently of lead-210, while in bone the initial uptake is of lead-210 and this is followed by quantitative retention of decay products.

Although this article is primarily concerned with polonium-210 it is of interest that at least four other isotopes of polonium, all α-emitters, are normally present in the human body: polonium-218 (radium *A*), polonium-216 (thorium *A*), polonium-214 (radium *C′*) and polonium-212 (thorium *C′*). Owing to their short half-lives, direct measurements of the tissue levels and distribution of these isotopes have not hitherto been possible, but experimental data obtained with rats[23] indicate that a dose rate to the kidney, due to polonium-218 and polonium-214, of the order of 1 mrad/year may result from continuous inhalation of radon at a concentration of 10^{-12} c./l. On the basis of the behaviour of polonium-210 the corresponding dose rate to the gonads may be expected to be of a similar order of magnitude.

Conclusion

The object of the work described in this article is to define the part played by polonium-210 in contributing to the biologically effective radiation dose received by man in a variety of environments. In the foregoing I have reported the gross concentrations of the nuclide that are found in human tissues, together with some of their variations with environmental conditions, and I have discussed the origin of the material and some information relating to its physiological and biochemical behaviour which suggests that its distribution within a tissue may be rather non-uniform. One major area of ignorance with which we are still faced, however, is in the basis for a comparison of the physical dose rates of high linear energy transfer radiation that we have measured with other,

predominantly low linear energy transfer, sources of background radiation; in other words, the problem of relative biological effectiveness at very low dose rates. In the absence of better evidence we may simply assume a relative biological effectiveness of 10 for the polonium-210 α-particle, in which case, by a convenient arithmetical accident, our tissue concentrations expressed as pc./kg become equivalent to mrem/year. This is almost certainly a very poor approximation to whatever the true position may be, and it should be pointed out that some recent reports[24-26] have suggested that the relative biological effectiveness for high linear energy transfer radiation may become very large at low dose rates, tending towards values of 30 or more for the conditions of background radiation. On this basis polonium-210 would evidently constitute a major fraction of background dose to man, the total of which is generally considered to be of the order of 100 mrem/year.

I thank Dr. C. F. J. Baron, Dr. D. Haler, Dr. L. A. Barrer and Dr. F. D. Sowby for arranging the supply of post-mortem material; Prof. K. Liden, Dr. J. K. Mietinnen, Dr. O. Paakola and Dr. P. M. Bird for the Arctic specimens; Prof. J. E. Roberts for the camel bones; Dr. I. W. Hughes and the British American Tobacco Co. for help in the experiments on cigarette smoking and tobaccos; and Dr. G. A. Stewart, of Messrs. Burroughs Wellcome and Co., for assistance with the measurements on insulins. I am indebted to several of my colleagues for their helpful advice, and to Mr. Howard Dawson for carrying out most of the measurements. Finally, I thank Prof. W. V. Mayneord for his support and advice.

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PATTERN MEASUREMENT

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PATTERN measurement is a rapidly growing development which is likely to affect a number of very different fields of study to a much greater extent than has previously been the case. The term is used here to describe any technique in which an instrument (usually of an electronic and optical nature) is used to scan an image or a permanent photographic record of an image, store the information obtained from the scan and finally analyse this information in terms of a series of parameters the quantitative values of which describe the features of the image which are essential, or at least of interest.

Clearly such instruments merely carry out essentially the same function as the combination of the human eye and part of the human brain. Indeed, those engaged in the field of pattern measurement develop a very healthy respect for the flexibility, speed and selectiveness of the human eye and brain in recognizing patterns. Unfortunately, as we all know, the human eye and brain are subject to fatigue; the brain is easily distracted and finds it difficult to remember with complete clarity the details in different parts of the same photograph and in comparing a series of similar photographs.

Bearing in mind the enormous capacity of a photograph to record permanently and conveniently the essential data in an image, it is understandable that most instruments in pattern measurement are designed to scan photographs rather than the original image.

Progress at Strathclyde

Nearly three years ago research and development were begun here to produce a series of instruments for pattern measurement. After a number of abortive starts using electro-mechanical devices of a fairly elaborate nature, the first of these instruments has been completed.

The problem in all instruments of this type, or at least the main problem, is to build into the instrument a very high degree of stability—short-term stability over periods less than a second and also long-term stability over periods of the order of days, weeks or even months. Unless such stability is present the advantages of the instrument over the human eye and brain are largely lost. There is simply no room for erratic unknown errors in the three processes of scanning, storage and analysis.

Other factors involved include the total number of points into which the photograph can be divided, the geometrical shape of the scan, the range of grain densities that may be encountered, the speed of the scan, the speed, capacity and permanency of storage of information obtained from the scan, the speed of extraction of this information and the flexibility and speed of its analysis. This does not exhaust all the practical factors which are encountered; for example, it is quite important to have visual monitors at different parts of the system, not only to check for satisfactory operation but also to permit satisfactory adjustment of the system to deal with particular types of images. To satisfy the demands of stability, speed and flexibility we have found it necessary to develop purely electronic/optical systems.

The particular instrument described here (Mark 1) is made up of three units which carry out the functions of scanning, storage and analysis respectively. The scanning unit, which will be described in a little more detail later, is an original instrument designed by ourselves in collaboration with General Electric Co., Ltd., who built it for us using modern, fully transistorized circuits. The storage unit is a Laben 512 channel analyser used in its multiscaler mode of operation, and we exploit three of its facilities for recording the data stored in its magnetic memory unit. These three facilities are a paper-tape puncher, an electric typewriter and a 'Polaroid' camera, which photographs a convenient display of the stored data. The analysis unit is a Ferranti *Sirius* computer.

Scanning Unit

Returning to the scanning unit, this is essentially a flying-spot scanner in which a raster of successive spots of the face of a cathode-ray tube is focused on a transparency (the photograph being examined) in the form of a 35-mm slide. Each spot had a duration of 1/10 of a second, and a measuring photo-multiplier behind the transparency provides a current which is proportional to the amount of light passing through the particular point on the transparency. Circuitry in the instrument converts this analogue current into a digital output which can vary between 0 and 128 depending on the grain density at the point being examined. The darker the film at a point the smaller is the integer which represents the measure of the grain density at this point. The instrument therefore accommodates 128 shades of grey and facilities are provided for varying over wide limits the range of grain densities corresponding to these 128 shades. The lower limit of grain density corresponding to 128 can be varied independently. The raster of points on the face of the cathode-ray tube can have from 4 to 512 points on each line and from 4 to 256 lines in each complete scan. The photograph being examined can therefore be divided into a total number of points varying from 16 to rather more than 125,000. The size of the raster can be varied electronically, thus providing a convenient method of continuously variable magnification in the scanning procedure. The area being scanned can be varied over a factor of 256. For convenience in setting up operations, prior to the actual scanning procedure, a display cathode-ray tube is built into the instrument which provides a reproduction of the selected area of the photograph. This reproduction looks very like the picture obtained on a television monitor. A line grid drawn on the face on this display tube makes it possible to correlate what is seen visually on the display tube, when the instrument is used in the viewing mode of operation, with the output on, say, the paper-tape puncher or visual display of the results of a scan as stored by the Laben 512 channel analyser. As it is possible to move the raster independently in the vertical or horizontal directions, a particular part of the display seen in the viewing mode of operation can be selected for scanning. Much more detailed information on this instrument will be given elsewhere, as it embodies a number of circuits entirely concerned with the problem of stability. However, it may be worth mentioning here that the instrument is driven by a master oscillator in both the high-speed viewing mode of operation and in the slow-speed scan, and a second photo-multiplier is used which monitors continuously the brightness of the spot of light on the cathode-ray tube (which provides the raster) and through feed-back circuits ensures that the amount of light in the spot focused on the transparency remains very constant.

In using the mark I model for pattern measurement a total of thirty 35-mm slides can be loaded into the scanning unit and examined separately one after another. Having viewed a selected slide by operating the scanner in the viewing mode of operation, it is then scanned in a series of 512 points. The 512 integers, varying between 0 and 128, corresponding to the digital output of the scanning instrument, are stored in the magnetic memory unit of the Laben 512 channel analyser. After visual inspection of these 512 numbers of the display tube of the Laben, they are printed-out on the paper-tape puncher. These operations are repeated until the whole of the selected area on one 35-mm slide has been scanned, and stored permanently in the form of the paper-tape print-out. This entire procedure can then be repeated for successive 35-mm slides, the paper tapes being inserted into the Ferranti *Sirius* computer which we programme to analyse the data.

Applications

Clearly this technique can be used to measure patterns in a series of photographs, or even just one photograph, in a wide variety of fields ranging from studies of chromosomes, a comparison of cancer and normal cells (see pp. 485 and 486 of this issue of *Nature*) to more detailed examination and classification of astronomical objects such as galaxies, Sun spots, the surface of the Moon and so on. These are the projects under way here at present, but it has been said that if an object can be photographed at all or if certain characteristics in a problem can be recorded photographically (for example autoradiographs or sweeps on a cathode-ray tube), then a suitable choice of a pattern measurement instrument may be of considerable use in extracting essential features of interest from the mass of data which are so easily recorded by a photograph. There is little doubt that instruments for pattern measurement will displace the unsupported human observer more and more as the years pass and techniques of measurement and problems being tackled become ever more ambitious and more sophisticated.

As an illustration of the operation of the instrument described here, Fig. 1 shows the result of a line scan across a photograph of the spiral nebula *M*51. Fig. 2 shows the corresponding appearance of the nebula as observed on the cathode-ray tube of the scanning unit operated in the viewing mode. The variations in grain density across the photograph of the galaxy are very striking. They show how all the information recorded in such a photograph can be transformed into an array of integers which can be permanently stored in the form of the paper-tape print-out and analysed at our convenience according to whatever programme is selected for the computer.

General Remarks

Perhaps the best way to think about pattern measurement is to consider the data, when inserted into the analys-

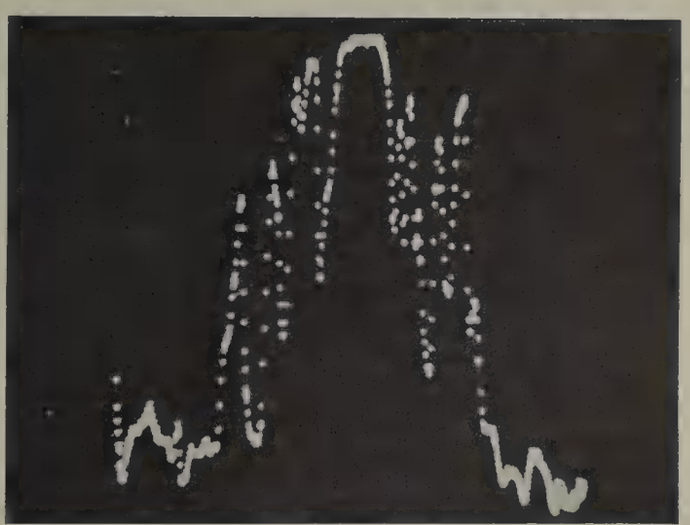

Fig. 1

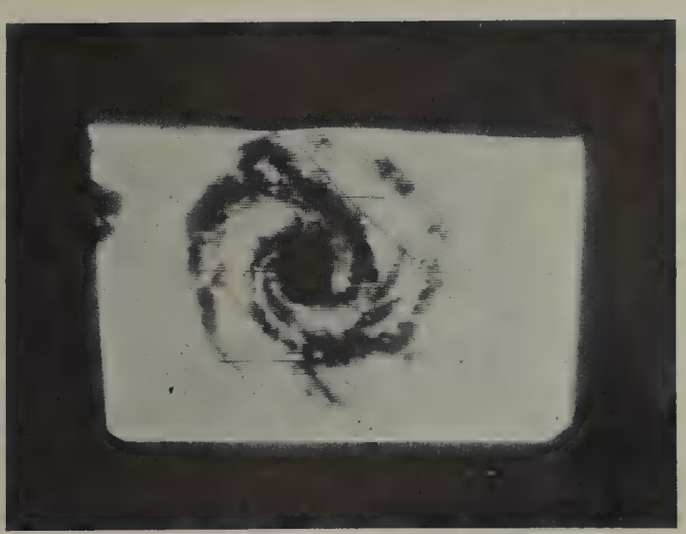

Fig. 2

ing computer, as a three-dimensional model of the original photograph or image. The original ordinary two dimensions are preserved by the geometry of the scan and the third dimension is the measured grain density. The function of the programme inserted into the computer is to 'look' at this three-dimensional model in a quantitative analytical way and measure values for parameters of interest. The word pattern reflects the fact that whatever the image is it will have a pattern which can conveniently be described in terms of a series of parameters which can be measured. The weakness of the human eye and brain is their inability to make precise measurements on these parameters and remember them sufficiently accurately for purposes of comparison. However, at least at present, the technique of pattern measurement still depends on the remarkable ability of the human eye and brain to recognize qualitatively the existence of parameters to describe the pattern of interest and thus make it possible to set up the appropriate programme for insertion in the computer.

Although devices for pattern measurement are not new developments, it is probably true to say that fairly recently, since the development of computer-type transistorized circuits and their widespread use, a number of instruments have appeared or are being developed which raise quite exciting possibilities when one considers, for example, the wealth of beautiful photographs obtained with great care and labour and now stored away in laboratories throughout the world. In fact, the development of pattern measurement makes it even more worth while straining to obtain even better photographic records.

We shall be pleased to hear from laboratories, here or abroad, interested in the possibility of collaboration in applying our instruments for pattern measurement to an analysis of the data recorded on existing or projected photographs.

It is our intention, funds permitting, to continue with the development of successive models with even wider facilities, faster speed of scanning and specialized computer and inspection facilities built into the original instruments. In this field, the continued development of genuinely useful instruments depends on the feed-back of experience gained by practical applications.

As a result of such feed-back we have come to the conclusion that, in general, there is no need to consider really large generalized computers even when pattern measurements are being made on complex images which require very large numbers of points to describe them adequately. The use of such computers is really only necessary if one insists on inserting all the information obtained, by scanning a complete image, before commencing the process of analysis. A study of our programme using the mark I instrument suggests strongly that it is worth considering pattern measurement instruments in which one, two or perhaps three lines only are scanned at a time and stored temporarily. This would be followed by immediate analysis using small specialized computer units to extract provisional values for the parameters of interest. These parameters are relatively few in number compared with the total number of points on one or two lines, and the provisional (or line) values of the parameters can be stored for subsequent analysis by a small final computer unit when all the image has been scanned and all the provisional values obtained. This separation of the computer analysis into two parts, provisional and final, is the major difference in our own work and similar efforts in the United States. It does not seem to introduce any great limitations for most images, it avoids the expense of time on large computers which can include the rental of land lines. Perhaps best of all, it opens up the real possibility of relatively inexpensive pattern measurement instruments for individual laboratories where the entire operations and the results are immediately in the hands of individual workers, thus allowing the all-important changes and adjustments which occur in any practical developing project.

This and other points are included in the projected instruments, now being designed here.

We acknowledge the provision of funds by the Department of Scientific and Industrial Research which enabled us to pursue this work, and the award of a D.S.I.R. research studentship to one of us (G. W. McM.). We also thank Mr. Donald Chadney of General Electric Co., Ltd., for his skilful choice of circuitry and components in the design and the successful construction of our present mark I instrument.

OBITUARIES

TWO PIONEERS OF PEST CONTROL

BY strange coincidence the two scientists who had the greatest influence on the development of the initially independent firm of Pest Control, Ltd., died within a few months of one another. Dr. W. E. Ripper crashed in the snow-covered mountains of Greece on March 21, 1965, while on a solo flight, though this fact was not known with certainty until his remains were discovered in June. Dr. E. Parry Jones died in hospital after a long illness, following a longer period of failing health, on June 30.

Both were trained as entomologists. Both gave the best years of their lives to control of pests in agriculture and particularly through their service to the same Company. Their abilities, service and successes were largely complementary. Both brought to bear talents which are essential for the development of a technically based industry. Ripper was essentially an initiator, a creator; sometimes ruthless, sometimes careless under his powerful inner drive to get things going. Parry Jones appeared far more conservative, but this was largely the response of a strongly self-disciplined man to the circumstances he had to face. Ripper's policy had brought the Company into a difficult financial position, and Parry Jones was insistent that, in the next phase of development, novelty and originality had to be kept within the framework of the commercially practicable.

The nature of the untimely death which overtook these men is in a strange sympathy with this difference between them. Ripper's friends had always advised him to forgo his passion for solo flying owing to deterioration of his eyesight, but he was an irrepressible enthusiast for physical as well as mental action, a keen skier and water-skier. He would perhaps have preferred to leave this world suddenly at the height of his powers. Parry Jones, also a keen sportsman in his younger days, had known himself to be in declining health for a long time owing to serious failure of lung capacity. He would not openly admit it, certainly never make a fuss about it, and strongly resented any tendency on the part of his colleagues to do so, but it was clear that he carefully conserved his physical energy and knew that he had to do so. Perhaps the discipline he had to apply to himself helped him to apply a similar discipline to the Company which he nursed round, after its acquisition by Fisons, Ltd., to become Fisons Pest Control, Ltd., from a position of considerable financial loss to one of sound financial profit.

These two men, in their different ways, were leaders in the field of applied science. Without the one the Company of Pest Control would never have started. Without the other it would not have survived the problems of adolescence. The death of both will be a great loss to many.

Dr. W. E. Ripper

Walter Eugen Ripper was born on January 24, 1906, in Austria, the son of Ministerialrat Max Ripper. He was a precocious child and a lot of his stimulus to scientific enquiry came from his father. He once told me that his earliest scientific publication was produced in his teens. He joined the United States Department of Agriculture as entomologist in 1931, working in Europe on the study of natural parasites for the control of pests introduced into the United States. He also acted as consultant to several large seed-growing estates in Austria and Hungary, devising special machinery for spraying unusual crops.

He realized that much good entomological research was being frustrated by the fact that only growers of very expensive crops could afford the necessary spray equipment. In 1938, at a congress in Berlin, he met Sir Guy A. K. Marshall, director of the Commonwealth Institute of Entomology, who was worried by the same difficulty. As a result, Walter Ripper settled in Cambridge, and in 1939 founded Pest Control, Ltd., of which he was managing director and Sir Guy was chairman.

Their aim was to bridge the gap between research and farming by providing a complete service, including machines, chemicals, labour and technical advice. By training, experience and temperament, Ripper was probably better equipped than anyone in Europe for such an enterprise. A man of tremendous physical and mental energy, he worked from 8 a.m. to midnight, seven days a week, driving himself sometimes to the verge of breakdown. A similar pace was expected of his staff, and it is a tribute to his power of inspiration that he was not disappointed.

For many people his drive to apply and exploit obscured an insatiable appetite for knowledge as such. Perhaps I saw more of this facet of a complex man than most. Except in the quiet *tête-à-tête* he would rarely admit ignorance, except, quite clearly, to himself, and then he would always take steps to correct it.

Ripper was always very quick to see the practical application of new research developments. In 1940 he introduced the weedkiller DNOC, which became mainly responsible for the disappearance of the picturesque sheets of red poppies which used to disfigure East Anglian cornfields. His first contract service in England was for the control of aphids by means of a portable nicotine fumigator. This gave a hitherto unobtainable degree of control, and without harm to predacious insects.

When the results of German research became available after the Second World War, Ripper was the first to seize on the idea of systemic insecticides, which could achieve selectivity and access to hidden insects without the need for special machines. His first introduction, 'Schradan', was also selective, leaving predators to 'mop up' aphids which might survive to give rise to resistant strains. Ripper always thought in terms of 'tailor-made' insecticides, each designed to kill one pest selectively. However, the increasing strictness of regulations for the use of insecticides and the sheer cost of obtaining official approval for a product of relatively limited market forced him to abandon this ideal.

Bringing research results to the farm was very often an engineering problem, and Ripper devised many special machines for the purpose. Even before helicopters became available for civilian use he began to consider their use as air-borne spray machines, and was the first to develop commercial aerial crop-spraying in the United Kingdom.

Shortly after the take-over of his Company by Fisons, Ltd., in 1953, Ripper severed contact with it. It was rather inevitable that the impatient initiator should not be in sympathy with the conservative policy the new management felt necessary to adopt. He joined the Dow Chemical Co., with whom he had had many previous contacts, to found their British subsidiary, Dow Agrochemicals, Ltd., in King's Lynn, near to his second wife's large farm at Docking, on which he carried out many experiments. He left Dow to return to his interests in contract spraying, founding Crop Savia, Ltd., in the Sudan, and pursuing further the possibilities of 'robot' aircraft for crop spraying. At the time of his death he was giving much thought to the careful, technically supervised, use of insecticides to avoid undesirable side-effects, a subject that had always been of intense interest to him.

He married first, in 1938, Berthe Siedeck, by whom he had a son and two daughters, and second, in 1952, Nancy Deacon. His son and his second wife intend to continue some at least of his uncompleted work.

Dr. E. P. Jones

Dr. Elwyn Parry Jones, or "P. J.", as he was invariably known to his friends and colleagues, was born in 1907 in Barry, Glamorgan, and educated at Taunton School. He obtained a first-class honours degree in zoology at the University of Wales and continued his education at the Imperial College of Tropical Agriculture, Trinidad, and at the University of Edinburgh, where he gained a Ph.D.

After a short period as a lecturer in the University of Aberdeen, he was seconded to the British South Africa Co. as a research entomologist at the Mazoe Citrus Experimental Station, Rhodesia, where he worked on the control of pests and diseases of citrus crops, until the beginning of the Second World War. After service in the Royal Air Force in Rhodesia, he was invited by Ripper to become the first managing director of a newly formed subsidiary company of Pest Control, Ltd., in Africa, and he was responsible for getting this Company, Pest Control (Central Africa), Ltd., off the ground.

The major activity of this new Company was control of pests of the tobacco crop, but it was also concerned with maintaining public health, and under Parry Jones's guidance it grew and thrived. Being closely associated with tobacco growing, he came to realize that there was an urgent need for a research centre to study improved pest control techniques if the industry was to survive and prosper, and he succeeded in interesting the Rhodesian Government and the Rhodesia Tobacco Association in the formation of the Pest Control Research Scheme, in which his Company was a partner. This arrangement led to the setting up of the present Tobacco Research Board.

Although he was keenly interested in African affairs, he declined an invitation to enter Rhodesian politics, feeling he could serve the country and the pesticide industry better in the Company.

When he returned to the United Kingdom in 1953, he became a member of the board of the parent Company and, shortly afterwards, it was taken over by Fisons, Ltd., and became Fisons Pest Control, Ltd. In the same year, he was made technical general manager, and in this position he was responsible for the research work carried out at Chesterford Park Research Station, where important chemicals used in farming have been developed.

In 1958, Parry Jones was made joint managing director, and in 1961, chairman and managing director. He was also chairman of Fisons Farmwork, Ltd., a member of the Fisons group main board and of the board of Fisons International Division.

He believed in long-term thinking, with an emphasis on a high level of research, and he considered the well-being of the staff to be of the greatest importance. He attached a high priority to education, and he took a great interest in the instructional courses organized by the Company for farmers, merchants, agricultural workers and advisers.

He saw the Company through its most difficult period. Wasteful marginal activities were pruned. Engineering research and development were abandoned, although the

contracting organization, separately managed as Fisons Farmwork, Ltd., was prosperously maintained. Concentration was on chemicals. Realizing that the search for new chemicals is becoming increasingly difficult and costly, he followed through the initiative of Avison Wormald, the first managing director under the Fisons régime, in negotiating development agreements with other organizations. Most important was the link-up with the Swiss giant, J. R. Geigy S.A., but this was followed by arrangements with Kureha Chemical Industries, Ltd., of Japan, and Boots Pure Drug Co., and successful licences were obtained from others for compounds which his own Company was well fitted to exploit.

Dr. Parry Jones leaves a widow, two sons and a daughter.

G. S. Hartley

Prof. K. R. L. Hall

Prof. Ronald Hall's untimely death on July 14 of this year, at the early age of forty-seven, came as a shock to all who knew him, and not least to his colleagues in the Department of Psychology, in the University of Bristol, where he had been professor since 1959.

During the six years in which Prof. Hall held the chair in the University of Bristol, he had built up an extensive research programme for the comparative study of social behaviour in primates. He was especially interested in early learning and the development of social behaviour in monkeys and baboons. He wrote extensively on the comparison between monkey and ape behaviour, and felt strongly that, since the early writings of people such as Zuckerman and Carpenter, so little had been done to further field studies of a comparative type. He himself was as much concerned with laboratory experiments as with field studies, and he was also concerned with the systematic accumulation of data from all sources to contribute to a picture—detailed in depth—of the behaviour of the animals who come nearest in human evolution to man.

Prof. Hall's work ranged from detailed studies of a specific kind, such as the social vigilance behaviour in the Chacma baboon, to a study of the sexual activity of the same animal, and on to more general efforts to build up an integrated picture, ultimately, of human behaviour. It was the problems of human behaviour that, in the end, he was working to unravel. His interests in animal studies dated from his period as professor of psychology at the University of Cape Town, which lasted from 1954 until 1959, when he returned to Bristol. Since 1959, he had achieved an international reputation for his work, and had by now published extensively in a wide range of journals. During 1962–63, he was a Fellow at the Centre for Advanced Study in the Behavioural Sciences at Palo Alto, California. He returned to East Africa in 1963 to carry out further ecological and behavioural studies of monkeys, and to build more detail into his expanding theoretical framework of behaviour.

Kenneth Ronald Lambert Hall was the only son of Kenneth Lambert Hall, a former acting Governor and Commander-in-Chief of Nyasaland. He was educated at Cheltenham College and at Brasenose College, Oxford, where he took his first degree in Law. During the Second World War, he served in the Middle East and in Germany as a captain in the Royal Artillery, and later as a staff officer. After the War, he returned to Oxford to study psychology, and was awarded the degree of D.Phil. in 1949. In the same year he was appointed head of the Department of Experimental and Clinical Psychology in Bristol Mental Hospitals and was also part-time lecturer in the Department of Psychology at the University of Bristol. During this period his interests were primarily in experimental abnormal psychology, a field in which he built a considerable reputation before his appointment in Cape Town, and his change of primary interest to his ethological work.

He was an exceptional athlete, and as an undergraduate played rugby football for the Oxford University Greyhounds and was awarded a blue for boxing. He continued to play cricket, tennis, squash and golf until his recent illness.

His loss is inevitably a great blow to the development of ethology, and the understanding of animal behaviour, especially as viewed as a basis for a comparison with, and understanding of, human behaviour. He leaves behind him, however, a collection of substantial publications which must in the course of time be brought together into a single collection of his life work.

In 1941 he married Pauline Sophie Assinder, who survives him.

F. H. George

NEWS and VIEWS

Science Education Officer, British Council : Mr. D. G. Chisman

Mr. D. G. Chisman, education officer of the Royal Institute of Chemistry, has been appointed to a new post of science education officer at the London Headquarters of the British Council from January 1966. The Council is increasingly being called on to provide assistance in the expansion and reform of science teaching in schools overseas, particularly in the developing Commonwealth. It now has six science teaching specialists in East and West Africa whose function is to provide assistance with the reform of the curriculum and methods, and the adaptation to local needs and conditions of the results of recent and current British work in this sphere. The new post will serve as a point of reference for the Council's overseas officers, keep them in touch with up-to-date British trends and supply information on new equipment, text-books and audio-visual aids, as well as on new teaching methods. Mr. Chisman, after graduating in chemistry at King's College, London, took a postgraduate certificate of education, and had some years practical secondary school teaching experience before joining the Royal Institute of Chemistry. Since then, as a secretary to the British Committee on Chemical Education and as a member of both the Consultative Committee for the Nuffield Chemistry Project and the Physical Science Committee of the Association for Science Education, he has been closely concerned with the study of the problems of school science teaching in Britain, and has also been involved with overseas problems as a consultant to the Organization for Economic Co-operation and Development and Unesco.

Zoology in the University of Newcastle upon Tyne : Prof. A. D. Hobson

Prof. A. D. Hobson retires from the chair of zoology in the University of Newcastle upon Tyne in September 1966, and is spending most of the year 1965–66 on sabbatical leave. He has been professor of zoology at Newcastle and director of the Dove Marine Laboratory since 1932, first under the title of Armstrong College, then King's College, and, since 1963, as the separate University of Newcastle upon Tyne. During his time at Newcastle he has seen the Department grow from a stage when it consisted of three lecturers, one of them half-time, to its present size of sixteen teaching staff. The closely associated Dove Marine Laboratory has also expanded during this period.

Prof. Hobson's early work was in experimental cytology and he was one of the group of young biologists concerned with the founding of the Society for Experimental Biology. During the Second World War, he worked on nematode physiology, particularly on cultural methods for *Ascaris*; his scholarly work on this subject is well known. Prof. Hobson's other great interest has been marine zoology and, in addition to his directorship of the Dove Marine Laboratory, he has been a member of both the North-East and the Northumberland Sea Fisheries Committees. Prof. Hobson has worked devotedly for the welfare of the Department of Zoology at Newcastle during this period of expansion and change. Many former students and colleagues in zoological work all over the world will wish him every happiness in his retirement.

Prof. R. B. Clark

Dr. R. B. Clark, who has been appointed to succeed Prof. A. D. Hobson, will carry on the tradition of work in marine biology so long established at the University of Newcastle. Dr. Clark started his career as a physical scientist, graduating from Chelsea Polytechnic in 1944 and taking up a post as scientific officer in the Road Research Laboratory of the Department of Scientific and Industrial Research. In 1947 he entered University College, Exeter, to read zoology with Prof. Harvey, gaining his special honours degree in 1950. From 1950 until 1956 he was assistant lecturer at Glasgow, though his tenure there was interrupted by periods spent in the University of Washington, Seattle, and at Berkeley, where he was assistant professor in the University of California from 1953 until 1955. He was appointed lecturer in zoology in the University of Bristol in 1956, and gained his London D.Sc. in 1965. His special interests in marine zoology have centred round the annelids, with particular reference to the polychaetes. Within this group, however, his work has ranged widely. He has contributed substantially to ecology and natural history, and his investigations on polychaete morphology undoubtedly stimulated his interest in the physical factors concerned in the evolution of invertebrates—illustrated in his recent book *Dynamics in Metazoan Evolution* (Oxford, 1965). Dr. Clark is probably best known for his extensive contributions to annelid neurosecretion and neuroendocrinology, where his work has been almost that of a pioneer in annelid physiology. More recently he and his students have published several papers on behaviour in polychaetes, with particular reference to the learning process. His interests will therefore admirably complement and extend those of the active group of zoologists now in the Department.

Metallurgy in Brunel College, London : Prof. C. Bodsworth

Dr. C. Bodsworth, at present chief physical metallurgist at the Central Research Laboratories of Richard Thomas and Baldwins, Ltd., has been appointed to the newly established post of professor and head of the Department of Metallurgy at Brunel College of Advanced Technology. His early industrial training was with the United Steel Co., Ltd. In 1950, after three years War service with the Royal Corps of Signals, he graduated in metallurgy at the University of Sheffield. He was awarded an M.Met. in 1951 and a Ph.D. in 1957. From 1952 until 1964 he was a lecturer in the Department of Metallurgy in the University of Liverpool. Dr. Bodsworth's scientific interests have centred on metallurgical thermodynamics, and he has written two books on this subject. He has also been actively engaged in investigations of various aspects of phase transformations in ferrous materials.

Assistant for U.S. Medical Research Fellowship Analyses : Dr. E. C. Bracken

Dr. Everett C. Bracken has been appointed assistant to the Chief, Research Fellowships Branch, National Institute of General Medical Sciences, National Institutes of Health. As professional assistant to Dr. Frederick P. Ferguson, Dr. Bracken will be responsible for conducting analyses of National Institute of General Medical Sciences fellowship programmes to evaluate their effectiveness and to identify scientific areas requiring special emphasis. For the past six years, Dr. Bracken has been at the University of Oklahoma School of Medicine, serving as associate professor in the Microbiology Department since 1960, and as associate professor in the Pediatrics Department and director of the Infectious Diseases Laboratory, Children's Memorial Hospital, since 1959. Dr. Bracken was previously at Vanderbilt University, where he was an instructor and assistant professor in the Microbiology Department of the School of Medicine from 1954 until 1959, and a teaching assistant and instructor in the Biology Department from 1951 until 1954. He is a member of the American Society for Microbiology, the New York Academy of Sciences, the Electron Microscopy Society of America, the American Society for Experimental Pathology, the Southern Society for Pediatric Research, Phi Beta Kappa, and Sigma Xi.

Minister of Power's Advisory Council on Research and Development

Mr. F. Lee, the Minister of Power, has appointed Sir Charles Sykes to the chairmanship of his Advisory Council in Research and Development. Sir Charles Sykes succeeds Lord Fleck, who has served as chairman of the Advisory Council since it was constituted in July 1960 and is now retiring. Sir Charles Sykes became a member of the Advisory Council on Research and Development on May 27, 1965. He is managing director of Thos. Firth and John Brown, Ltd., and chairman of Firth–Vickers Stainless Steel, Ltd. He served as a member of the Advisory Council for Scientific and Industrial Research from 1954 until 1956 and as a member of the Research Council of the Department of Scientific and Industrial Research from 1962 until its dissolution in March 1965. Lord Fleck served as chairman of the Minister of Power's Scientific Advisory Council from 1958 until 1960.

The terms of reference of the Advisory Council are: (1) To advise the Minister of Power on research and development in relation to his statutory duty of securing the effective and co-ordinated development of coal, petroleum and other sources of fuel and power in Great Britain, and of promoting economy and efficiency in the supply, distribution, use and consumption of fuel and power, whether produced in Great Britain or not. (2) To advise the Minister of new scientific and technical knowledge or applications of knowledge throughout the world, which in the opinion of the Council should be taken into account in the performance of his statutory duties. (3) To keep the whole field of fuel and power under continuous review with the object of identifying problems needing research and development and advising the Minister of these problems with the view of discussion with the industries concerned.

Other members of the Council are: Captain (E.) W. Gregson (deputy chairman), Mr. M. A. L. Banks, Prof. P. M. S. Blackett, Sir Charles Cawley, Mr. H. E. Collins, Sir Kenneth Hutchison, Sir Harry Melville, Mr. R. V. Moore, Dr. L. Rotherham and Prof. M. W. Thring. The assessors are Dr. E. Lee, Mr. H. T. Ramsay and Mr. H. S. Stephenson.

University Entrance Requirements in Britain

The third edition of *A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom, 1965*, which has been compiled from material provided by the universities to the Committee of Vice-Chancellors and Principals, has been extensively revised and is more comprehensive than either of its

predecessors (Pp. 219. London: The Association of Commonwealth Universities, 1965. 11*s*. 6*d*.). Its coverage of combined honours courses has been extended and also expanded to include details of the entrance requirements of the ten colleges of advanced technology and the Heriot-Watt College, all of which are to have the status of university institutions. In addition, the tables include for the first time the entrance requirements of the Scottish universities, expressed in terms of the General Certificate of Education. In this edition the course requirements of the principal full-time first-degree courses offered by the universities of the United Kingdom are set out in terms of the General Certificate of Education in eighty tables, covering single honours, combined honours and general degree courses. The information provided in the text is supplemented by a self-indexing list of combined honours courses offered by fewer than four universities, and by notes on particular institutions the courses and requirements of which do not fall into the usual pattern. The compendium is not an official publication of the universities concerned and cannot be regarded as superseding their own publications, which are the ultimate authorities. The tables indicate that many universities formally require only two Advanced Level passes in the General Certificate of Education for most courses in arts, law and social studies (and for a few in science and technology), whereas other universities formally require three. Most candidates present three or more, which many university selectors regard as preferable. However, the pattern of academic entrance requirements may still be unduly complex, and it has been agreed that consideration of ways in which general and course requirements might be simplified will be one of the first tasks to be undertaken by the Standing Committee on University Entrance, a delegate body, established in June 1965, representative of the universities of the United Kingdom.

La Trobe University, Melbourne

La Trobe University, which will be the third University in the Melbourne metropolitan area, was established under an Act of the Parliament of Victoria on December 9, 1964. The site for the new University is an undeveloped tract of some 500 acres about 8 miles north-east of the city centre. Planning of the site works and initial buildings is proceeding and the University will open in March 1967. Planning and development of the University, in the present preparatory stage, are controlled by an Interim Council of twenty-five members under the chairmanship of J. R. A. Glenn, chairman and managing director of I.C.I.A.N.Z., Ltd. The principal officers appointed so far are: *Vice-Chancellor*, Dr. D. M. Myers (at present dean of the Faculty of Applied Science, University of British Columbia); *Librarian*, D. H. Borchardt. Applications have been requested for foundation chairs in English, history, philosophy, a modern European language, economics, sociology, politics, mathematics, physics, chemistry and biology; but so far no appointments have been made. The academic work of the new University is to be organized in schools of related disciplines. Each school will be the functional unit for teaching, research and administration in the disciplines which it embraces. Its field will be wider than that of a traditional department but narrower than that of a faculty. Student activities will be centred on colleges that will provide study, recreational, social, dining and some tutorial facilities for all members, and residential facilities for a minority of the members. All members of the academic, library and administrative staffs will also belong to the colleges.

Museums Journal

The *Museums Journal* (65, No. 2; September 1965. 15*s*.) includes a number of interesting papers, in addition to a full report of the annual conference of the Museums Association in Dublin in June 1965. Dr. S. Dillon Ripley, as secretary of the Smithsonian Institution, describes the Institution as a great museum centre; Mr. C. Douglas Deane gives details of the Field Museum and Nature Trail in Northern Ireland; and Mr. H. Wakefield gives much useful information to curators concerning the availability of purchase grants from the Victoria and Albert Museum, London, to provincial museums. Mr. N. Cook, president and keeper of the Guildhall Museum, London, dealt with many subjects in his address, chiefly those concerning the Museums Association in relation to Government grants. Dr. A. T. Lucas, director of the National Museum of Ireland, developed the theme of the role of the National Museum in the study of Irish social history, and Mr. R. Rowe spoke on "Art Museums and the Idea of Progress". At the annual meeting, Sir Frank Francis, director of the British Museum, was elected president for 1965–66, when the annual conference will be held in Sheffield during July 4–9.

British Museum : Department of Western Asiatic Antiquities

The British Museum regrets to state that the Assyrian sculpture, mostly of Sennacherib and Ashurbanipal, from their palaces at Ninevah, which has been exhibited in the Assyrian Saloon and the Assyrian basement, will be for the most part unavailable for examination until further notice, owing to the rebuilding of the Assyrian Saloon and the insertion of a new floor.

Storage of Books

An essay by P. W. Plumb, entitled "Central Library Storage of Books", which was awarded the Sevensma Prize, 1962, has been issued as *Library Association Pamphlet* No. 24 (Pp. 57. London: Library Association, 1954. 1965. 16*s*.; L.A. members 12*s*.). It reviews experience gained in the United States, Britain and elsewhere, including the use of microfilm. There are brief appraisals of methods used at the British Museum Newspaper Library, the Storage Library of the University of London, the National Central Library and the National Lending Library for Science and Technology, as well as at the Bibliothèque Nationale Storage Library, and, in the United States, the New England Deposit Library, the Midwest Inter-Library Centre, and the Medical Library Center of New York. Mr. Plumb commends the systems of the National Central Library and the National Lending Library for Science and Technology, and concludes that a national scientific and technical lending library and one for the humanities and sociology in each country with similar but perfected aims and techniques would go far to solving the space problems of libraries. Certainly this suggestion, if applied, would help in avoiding the major disabilities apparent in many existing schemes and proposals.

Directory of Indian Scientific Periodicals

The *Directory of Indian Scientific Periodicals, 1964*, with its 725 entries listing periodicals current to the end of 1963, including annual reports of scientific and technical institutions and other learned societies, is intended as a first step towards consolidating and servicing scientific communication in India (Compiled by G. K. Arora, S. Dutta, D. N. Gupta and H. N. Rangachar. Pp. 133. Delhi: Indian National Scientific Documentation Centre, 1965). The *Nifor Guide to Indian Periodicals*, published at Poona in 1955–56, and a cyclostyled list of Indian scientific periodicals issued in 1960 laid some foundation for the present *Directory*. The entries are arranged according to the Universal Decimal Classification and there is an alphabetical index of titles. The periodicals are mainly in English, about half a dozen being bilingual with English as one language; there are 60 periodicals in Indian regional languages (33 in Hindi) and one in French. The *Directory* is well printed and well set out.

Volcanological Research in Britain

On behalf of the Volcanological Research Committee of the Royal Society, the terms of reference of which are "to encourage, promote and co-ordinate the comprehensive study of volcanicity . . .", a booklet has been published to record the scope of researches being undertaken at present by universities and other scientific institutions in Britain on modern and ancient volcanoes (*United Kingdom Volcanological Research, 1965*. A summary of current volcanological studies with provisional plans for future years. Pp. 55. London: The Royal Society, 1965). The extent of the studies recorded is wide, embracing work at about a hundred localities outside the British Isles, described in close on 150 recent papers (inclusive of some reports of researches in experimental petrology). It is hoped to publish a further edition of the booklet in about two years' time when attention will be restricted to current investigations on Tertiary and Recent volcanicity.

Age Determinations of North American Rocks

A compilation of the radiometric ages determined for rocks of the North American continent, complete up to May 1964, has recently been published by the Committee on Nuclear Science of the National Academy of Sciences—National Research Council in Washington (Publication 1276: *Geochronology of North America*. Nuclear Science Series—Report No. 41. Pp. v+315. Washington, D.C.: National Academy of Sciences–National Research Council, 1965. 6 dollars). In it, a brief introduction to the present status of geochronological methods, by Dr. G. W. Wetherill, is followed by a 270-page tabulation of results listed on a regional basis and by a bibliography of 144 items. The oldest well-dated rocks in North America bear ages of about 2,500–2,800 million years, and are found in an apparently continuous belt extending from Quebec through Ontario, Manitoba, Minnesota, North Dakota, Montana, and into Wyoming, as well as in the Great Slave Lake area of the North-West Territory. They are apparently much younger than the most ancient formations of southern Africa and of arctic Russia. Extensive recent data supplementary to the results in the American compilation have been published contemporaneously by the Geological Survey of Canada and are accompanied by a report by Dr. C. H. Stockwell which proposes a new time–stratigraphic classification and nomenclature for the rocks of the Canadian shield (Paper 64–17. Parts I and II. *Age Determinations and Geological Studies*. (I) Pp. iv+126. 75 cents. (II) Pp. iv+29. 75 cents. Ottawa: Queen's Printer, 1965). In this, the Archaean and Proterozoic are each identified as eons, with the latter divided into the Aphebian, Helikian (Palaeohelikian and Neohelikian) and Hadrynian eras, the lower boundaries of which are respectively marked by the Kenoran (2,390 m.y.), Hudsonian (1,640 m.y.; Elsonian at ?1,280 m.y.), and Grenville (880 m.y.) orogenies.

Soil Micro-fauna

The formation of humus is a most important matter to foresters, not only because it helps to maintain proper conditions for tree growth, but also because it may bring about improvements to the quality of the trees and give greater productivity to the site. A big part is played by oribatids in the breakdown of the litter and its conversion into humus, and members of this super-family of the Acarinae occur in very large numbers in the soil fauna. In *Contribution à l'Etude Ecologique des Oribates de la Litière dans une Forêt de Moyenne-Belgique*, by Philippe Lebrun, we not only have an example of a very fine piece of research carried out in 13 months of field work, but also results presented in a full and yet concise manner, and set out with proper reference to the other factors affecting the biocenose (Institut Royal des Sciences Naturelles de Belgique. Memoire No. 153. Pp. 96. Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1965). The published account is divided into three chapters. The first is an account of the locality factors. The research was carried out in an oak-wood on the south-eastern slopes of a small valley near Louvain in central Belgium in 1961–62. Information is given of the vegetation, soil, local climate, the water-holding capacity of the soil and the evapotranspiration. The second chapter is devoted to the synecology of the oribatids with the view of discovering the bonds existing between different groups of them. The final chapter deals with the autecology of the oribatids found on the site. The forester recognizes the great importance of these minute members of the animal kingdom, but it is not always remembered in what quantities they occur. In a Belgian oak-wood it is estimated that there are about 75,000 individuals per square metre of the upper soil and humus layers and even up to as many as 300,000 per square metre. Thus, their numbers alone present some difficulty to the research worker who, in this case, had to contend with 48 species in a sample population of 85,000 individuals. Philippe Lebrun has not only clearly described his fine piece of research but he has also taken into consideration, as is very proper but not always done, the various factors of the vegetation, soil and climate which contribute to the biocenose. Thus, the danger in such a work of omitting some important factor affecting the result has been avoided. The numerous histograms and graphs make for easy understanding of the various components of the investigation. Nothing seems to have been forgotten: for example, there is a histogram showing the precipitation reaching the soil compared with the actual precipitation. This is but one example of the detail to be found in a work which will be acclaimed by foresters and others interested in the soil micro-fauna.

Oil Pipeline over the Alps

It may not be generally realized that work is in progress on the colossal project of constructing a 40-in. diameter, 300 miles long, Trans-Alpine oil pipeline to convey oil from the Adriatic to the heart of Germany, as so far this scheme has received relatively little publicity. To a brief extent, this has now been remedied by an article entitled "Pipeline Over the Alps" (*Esso Magazine*, Esso Petroleum Company, London, Summer 1965). The route lies from Trieste, by Udine, Lienz, Kitzbuhel, Kufstein, Rosenheim, east of Munich to Ingolstadt. It necessitates the construction of three tunnels under the Alps, one known as the Plöcken Tunnel, between Udine and Lienz; one known as the Felber Tauern Tunnel, and another as the Hahnenkamm Tunnel, both between Lienz and Kitzbuhel. These are indeed major engineering feats in themselves, and the photographic illustrations included in the article, together with a panoramic map in colour, make this abundantly clear. This project will ultimately include the construction of a branch pipeline just north of the Plöcken Tunnel to link up with the Austrian State Refinery near Vienna. The Trans-Alpine pipeline is a venture in which several oil companies are taking part and in which the Esso Petroleum Company has the largest interest. However, because the pipeline has to pass through three separate countries, three separate companies have been formed for constructional and operational purposes in their respective areas. These companies are Deutsche Trans-Alpine Oelleitung G.m.b.H., Trans-Alpine Oelleitung in Osterreiche G.m.b.H., and Società Italiana per L'Oleodotto Trans-Alpino S.p.A. The German company is the co-ordinating company. Among the many practical problems concerned with such a project, apart from tunnelling and mechanical excavation in the high Alps, are the necessity to dredge the harbour at Trieste so that it can eventually accommodate oil tankers of 160,000 dead weight tons; setting storage tanks there on piles because

available land is a rocky hill site; construction of several thousand feet of piers into the Adriatic, so laying many connecting pipelines to the tank-farm. Involved also in the scheme is the building of five separate pumping stations, each equipped with two 4,000-horse-power electric centrifugal pumps required to lift hundreds of thousands of tons of oil from sea-level to one of the highest points at Felber Tauern. It would appear that completion of the main works and expectation that the new pipeline will be 'on stream' are likely in about two years' time, which will be a most remarkable achievement if realized.

University News:

Aberdeen

Dr. H. J. Evans has been appointed to the newly created chair of genetics. The following lecturers have also been appointed: Dr. P. G. Jarvis (botany); J. F. Robbie (engineering); Dr. J. B. Wilson (geology); Dr. G. F. Burnett (agriculture).

Manchester

Dr. D. M. McDowell has been appointed to the chair of hydrodynamics and hydrology. The following lecturers have also been appointed: Dr. G. N. Smith (chemistry); W. McLewin (mathematics); Dr. A. R. Mainwaring (pathology); Dr. H. A. Lee (medicine).

Birmingham

The following appointments have been made: *Lectureships*, Dr. C. J. Hooke (mechanical engineering); V. L. Moffat (engineering production); Dr. B. Sklarz (chemistry); Dr. J. H. Coote and Dr. A. R. Tindall (physiology); Dr. I. D. Green (medicine); Dr. N. R. Ling (experimental pathology); *Senior Fellowship*, Dr. J. M. Whittaker (pure mathematics); *Research Fellowships*, P. F. Adams, C. G. Drury and I. T. Franks (engineering production); K. J. Blackburn, C. J. Chesterton and J. Kendrick-Jones (biochemistry); S. F. Campbell, Dr. W. B. Hollyhead and M. A. Salam (chemistry); Dr. M. K. Das and Dr. M. M. Sadek (mechanical engineering); G. Immirzi and Dr. D. H. Lyth (mathematical physics); J. Jafar (physics); M. J. Pemberton (minerals engineering); M. W. Stanley (physical metallurgy); Dr. P. S. Hasleton (pathology); Dr. C. C. McCormick (experimental pathology).

Lalor Foundation Grants for 1966

The Lalor Foundation has announced the programme of awards for 1966, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open to all nationalities and may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff, with an upper age-limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere for appropriate projects in the fields specified. These awards will normally not exceed 1,200 dollars for a single man or woman, 1,400 dollars for a married man working at his home institution, and 1,550 dollars for a married man with a principal programme at another institution. Further information and application forms can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware. The final date for receipt of executed applications, complete with supporting data, is January 15, 1966. Notification of appointment will be on or before March 15, 1966.

European Society for Comparative Endocrinology

At the third meeting of European comparative endocrinologists, held in Copenhagen during August 2–5, it was decided to form a European Society for Comparative Endocrinology. The following were elected as the first officers and council of the Society: *President*, H. Heller (Bristol); *Vice-President*, M. Fontaine (Paris); *Secretary and Treasurer*, P. G. W. J. van Oordt (Utrecht); *Honorary Editor of the Proceedings of the Society*, E. J. W. Barrington (Nottingham); *Council Members*, H. Herlant-Meewis (Brussels), C. Barker Jørgensen (Copenhagen), M. Lüscher (Bern), V. Mazzi (Turin) and V. J. A. Novák (Prague). Those interested in the aims of the Society are invited to write to the Secretary, Zoological Laboratory, State University of Utrecht, Janskerkhof 3, Utrecht.

Announcements

Dr. H. E. Huxley has been awarded the first William Bate Hardy Prize of the Cambridge Philosophical Society for his work on the structure of muscle. The William Bate Hardy Prize was established in 1964 by the Cambridge Philosophical Society to commemorate the centenary of the birth of Sir William Bate Hardy, a Fellow of the Society from 1889 until his death in 1934. It is awarded for the best original memoir, investigation or discovery by a member of the University of Cambridge in connexion with biological science published during the three years immediately preceding the award. The present value of the Prize is £200.

A short course for librarians and information officers on "The Trade Literature Collection", arranged by Aslib, will be held at Aslib during November 25–26. Further information can be obtained from the Education Officer, Aslib, 3 Belgrave Square, London, S.W.1.

A seminar on "Creativity in Science", arranged by the Science of Science Foundation, will be held at the Foundation on December 8. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

The second Australian conference on "Hydraulics and Fluid Mechanics" will be held at the University of Auckland during December 6–12. Further information can be obtained from the Convenor of the conference, School of Engineering, Ardmore College Post Office, University of Auckland, Auckland.

The 1965 International Building Exhibition will be held at Olympia, London, during November 17–December 1. During the Exhibition, five conferences will be held: building for people; system building—can it be economic?; exporting building materials; the important role of building component manufacturers; the change to metric. Further information can be obtained from the Conference Secretary, the Building Exhibition, 11 Manchester Square, London, W.1.

A meeting of the Biochemical Society will be held in the University of Manchester during November 11–12. The programme will include a colloquium on "Biosynthesis in Micro-organisms: Genetic and Environmental Factors"; a discussion meeting on "Biochemistry and Industry"; and the Colworth Medal Lecture on "The Role and Control of the Glyoxalate Cycle in *Escherichia coli*", to be delivered by Prof. H. L. Kornberg. Further information can be obtained from Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff.

Erratum. In Table 4 of the article entitled "Identification of Dinitro-octylphenols in Certain Commercial Fungicides", by Dr. A. H. M. Kirby and L. D. Hunter, which appeared on p. 189 of the October 9 issue of *Nature*, the last five lines should be amended to read as follows:

7·41(7*q*)	7·37*q*	7·26*m*	7·15(6·8*se*)		1-CH
				7·28(7·5*t*)	1-CH$_2$
1·91*s*	1·89*s*	1·85*s* 1·88*s*	1·90*s*	1·87*s*	5-H 3-H

HORTICULTURAL SCIENCE AT THE UNIVERSITY OF READING

THE diamond jubilee of the Horticultural Education Association was marked by a conference at the University of Reading during September 6–9. The object of the conference indicated by the theme, "Horticultural Science at the University of Reading", was to bring together contributions from a number of the branches of science that find a meeting point in the technology of horticulture. It was part of the success of the conference that it directed attention to the importance of border-line research between disciplines and to the desirability of combining pure and applied research.

Contributions from the Department of Horticulture were concerned with plant physiology and plant protection. Prof. O. V. S. Heath discussed methods of measuring stomatal aperture and pointed to the importance of a knowledge of stomatal physiology in attempts to control water loss from crop plants. Dr. H. Meidner demonstrated a portable and relatively cheap instrument for measuring the water content of soils by determination of their heat conductivity. One of the main advantages of the method was that the probe employed, unlike the porous block used in other techniques of soil moisture determination, did not have to equilibrate with water in the soil.

Papers by Dr. D. Vince and Dr. G. P. Harris were concerned with the photoperiodic control of flowering in long-day plants. Dr. Vince described experiments with different strains and species of *Lolium* that demonstrated a changing sensitivity to red and far-red radiation during a 16-h night. It appeared that in order to produce a maximum promotion of flowering by interruption of the night with red light, the timing of the interruption had to be varied according to the species or strain of *Lolium* used. The potentialities of detailed investigations of the photoperiodic responses of plants of horticultural importance were underlined in the paper by Dr. Harris on flowering in the glasshouse carnation. Out of a number of photoperiodic treatments tested, the use of lighting from tungsten-filament bulbs throughout the night was found to be most effective in promoting flowering under winter conditions. Applications of this observation to commercial practice were discussed. Dr. H. F. van Emden bridged the gap between plant physiology and entomology in a discussion of plant–insect relations. Results were presented indicating the role of the nitrogen status of the plant and particularly the soluble-nutritional content in controlling rates of aphid multiplication.

The Agricultural Research Council Unit of Flower Crop Physiology, which, under the direction of Prof. O. V. S. Heath, is attached to the Department of Horticulture, was represented both in a comprehensive demonstration of equipment and in a paper read by Dr. A. P. Hughes. The main item of equipment in the Unit is 9 (later to be 12) growth cabinets designed by the National Institute of Agricultural Engineering and housed in a special hall with controlled ventilation. Dr. Hughes described the use of this equipment to investigate interactions of light intensity, temperature and carbon-dioxide concentration. He also presented results of experiments demonstrating effects of photoperiodic treatments on leaf expansion in a variety of plants and discussed the use of such treatments under conditions where light intensities are limiting to growth in dry weight.

In a contribution from the Department of Physiological Chemistry, Dr. K. A. Hassall discussed problems encountered in the use of crop protection chemicals including questions of toxicity, persistence and selectivity. Experimental results showed how the persistence and effectiveness of a pre-emergence weed-killer could vary with the amount of rain falling after application.

From the Department of Agricultural Botany there were papers on physiological ecology and plant breeding and genetics. Prof. A. H. Bunting described work on the water relations of plants growing on soils of the chalk downs near Reading and demonstrated the capacity of the chalk to retain water by reference to its *p*F-moisture content curve. The pore size distribution derived from this curve was shown to be similar to a distribution based on direct measurements of pore sizes in electronmicrographs. Dr. J. K. Jones and Mr. G. D. Rowley in contributions concerned with plant breeding both emphasized the value of using wild species as sources of genetic variation. Dr. Jones presented evidence that the sets of chromosomes in all species of strawberries were homologous, that it was possible to produce many inter-specific hybrids and to induce polyploidy and therefore to use most, if not all, of the species in breeding. Mr. Rowley showed how the derivation of cultivars of rose could be traced by crossing supposed parent species and underlined the importance of making provision for the conservation of species and cultivars that might not be of immediate commercial value.

This report indicates the varied nature of the papers presented; but the contributions had in common an immediate or potential value to horticulture. The conference provided members with the opportunity to examine the role of the University in the development of this subject.

G. P. HARRIS

THE WORLD HEALTH ORGANIZATION, 1964

A REMINDER that the control of communicable diseases is "still the most important health challenge facing mankind" is given by Dr. M. G. Candau, director-general of the World Health Organization, in his annual report for 1964 *.

It is not, therefore, surprising that, notwithstanding the new developments in the work of the World Health Organization, the chapter on communicable diseases remains the longest in the volume. While some diseases have declined in public health and economic importance, others are still obstinate or present new problems.

Dr. Candau points to the increasing incidence of plague the heavy toll of cholera and smallpox, the continuing problems of malaria and tuberculosis, the upsurge in the incidence of syphilis and gonorrhoea. He lists, as the three main obstacles to effectivecon trol, lack of knowledge, insufficient international support for the Organization and financing of campaigns and, most formidable of all, lack of adequate health services in the countries most affected. Lasting success can be achieved only through the establishment of permanent and effective health services applying national health plans.

The experience of the Organization has shown that quick results cannot be expected; the countries most in need of assistance for national health planning are those where

* *Official Records of the World Health Organization*, No. 139: *The Work of WHO*. Annual Report of the Director-General to the World Health Assembly and to the United Nations. Pp. xiv + 240. (Geneva: World Health Organization; London: H.M.S.O., 1965.) 4 Sw. francs; 6*s*. 8*d*.; 1.25 dollars.

statistics are scanty, the shortage of trained staff acute, and where multiple health problems compete for priority. Nevertheless, Dr. Candau is able to report progress in comprehensive health planning in a number of countries, for example, Africa, Latin America, and Asia—the areas presenting the greatest problems.

In campaigns against many of the communicable diseases, the use of research is a valuable adjunct to the work in the field. In its medical research programme, the Organization draws on the advice of leading experts in many special fields and now has the collaboration of laboratories in all parts of the world. The lists of the reference laboratories of the World Health Organization and of scientific meetings during the year provide some indication of the scope of the programme. The research programme is by no means restricted to the communicable diseases. The report contains, for example, accounts of the present studies on cancer (including the possible relation between viruses and cancer), cardiovascular diseases, and human genetics—a new field of activity for the World Health Organization (see *Nature*, **208**, 230; 1965). The importance of immunology is stressed, and achievements are mentioned in the Organization's relatively new programme of immunological research, particularly in relation to parasitic diseases—a hitherto largely unexplored area of immunization in which further research has been planned on the advice of an expert committee.

Other topics dealt with in the "General Review", constituting Part 1, include malaria eradication, environmental health, public health services, health protection and promotion, education and training, biology and pharmacology, health statistics, publications, information, and administration and finance. A final chapter outlines the work done in co-operation with the United Nations and related agencies, and with non-governmental organizations.

Part 2 deals with the health problems and programmes in each of the Organization's six regions—Africa, the Americas, south-east Asia, Europe, eastern Mediterranean and western Pacific. In each chapter, broad surveys of work in the region concerned are complemented by detailed descriptions of some of the typical projects in which the Organization is giving assistance. They include the preparation of a plan to be financed under the United Nations Special Fund for the metropolitan area of Accra in Ghana; the improvement of nursing education and services in Guatemala; a project in south-east Asia to increase the local production of freeze-dried smallpox vaccine, which is essential for the successful pursuit of the smallpox eradication campaign in tropical countries; malaria eradication in Pakistan; and, in the western Pacific region, the first regional conference of deans of medical schools. For the European region, one of the projects described is a symposium in Moscow on the toxicology of drugs and methods of ensuring the therapeutic safety of drugs.

The list of current projects contained in Part 3 is a reflexion of the Organization's varied activities and of their adaptation to the particular requirements of countries in the various regions.

Information given in the annexes includes membership of the Organization, its executive board and expert committees, the budget, the secretariat, fellowships, awards under the medical research programme, and the reference centres.

A COMPARISON OF EUROPEAN HEALTH SERVICES

ALL European countries have some form of administrative arrangements to deal with environmental health; for example, housing standards, water purity, sewage and refuse disposal, and food hygiene. Modern life has brought into prominence new environmental problems, largely arising from industrialization and urbanization. A special monograph on health services in Europe was prepared for the European Conference on Public Health Administration held by the World Health Organization Regional Office for Europe in Zagreb, Yugoslavia, in June 1964*.

Air pollution is one cause for concern. Already most industrialized European countries have special regulations giving local authorities a measure of control over industrial emissions. Czechoslovakia and other countries have established standards for the control of health hazards from this source, while in the Federal Republic of Germany there are trade regulations and a Civil Code. In the U.S.S.R., the Institute of General and Communal Hygiene of the Academy of Medicine is carrying out research on air pollution in the principal industrial centres. Maximum permissible concentrations for 20 pollutants commonly found in the air of industrial cities have already been worked out. In the United Kingdom, smokeless zones are being established under a Clean Air Act. In Spain, provision is made for air disinfection in hospitals and certain clinics, and special legislation has been created covering unhealthy and dangerous gases and smoke.

Noise, as a public health problem, is also being tackled. Bulgaria has imposed restrictions on it in industry and in transport. In Spain, what are called "silence campaigns" have been carried out in the larger towns, where noise elimination is the concern of the municipal authorities and industrial boards. The U.S.S.R. has set up a Central State Health Inspectorate to deal with problems concerned with noise.

In many European countries, in accordance with the requirements of the International Commission on Radiological Protection, radiation hazards have been the subject of precautionary measures. In the United Kingdom, watch is kept on radioactivity in the air and in the soil by the Atomic Energy Authority in collaboration with the Medical and Agricultural Research Councils, and the Ministry of Housing and Local Government supervises the disposal of radioactive material. In Denmark, under an Act which has been in force since 1953, no radioactive material may be manufactured, owned or imported without special permission from the National Health Service, acting on behalf of the Ministry of the Interior; the Act lists safety precautions covering transport, storage, and use. Finland's Radiation Protection Act controls X-ray and other radiation installations, and Norway also has regulations covering the protection of personnel and the transport of radioactive material. Similar provisions exist in Czechoslovakia, Hungary, Ireland, Switzerland and Turkey. In the Federal Republic of Germany, the Max-Planck Institute of Biophysics carries out systematic investigations of radioactivity in ground water, rivers, and soil, and the meteorological stations make regular measurements of radioactivity in the atmosphere.

To-day, all countries in Europe have some arrangements for controlling the spread of infectious diseases, and the incidence of most of them has been on the wane for some time. Tuberculosis, however, still remains a cause for great concern. In the Federal Republic of Germany, several *Länder* have legislation providing for regular

* A limited number of copies of this document (reference *Euro* 268–4) are available to persons officially or professionally interested in the subject, on request from the World Health Organization Regional Office for Europe, Copenhagen.

chest X-ray examinations. One result of this approach has been the discovery that the percentage of undiagnosed cases of tuberculosis is higher among older people. In Norway, the Ministry of Social Affairs has the power to enforce X-ray screening of part of, or all, the population, as well as to test for tuberculosis, and to give B.C.G. vaccination. The radiography services in Italy are aimed at detecting not only tuberculosis but also other respiratory diseases, cardiovascular diseases and lung tumours. Compulsory B.C.G. vaccination is practised in some countries, such as Yugoslavia. Tuberculosis is notifiable in most European countries, but not in The Netherlands, Portugal or Romania.

There is a good deal of variation in the regulations regarding immunization against such infections as smallpox, diphtheria, tetanus, and whooping cough. Smallpox vaccination is compulsory in most European countries, and it is prescribed by the International Sanitary Regulations. It is required, for example, in Czechoslovakia, Hungary, Turkey, the U.S.S.R., and Yugoslavia, but it is not compulsory in the United Kingdom. In Yugoslavia immunization against diphtheria and tetanus is also obligatory.

Some communicable diseases are confined to particular areas. In Europe, for example, trachoma is endemic only in the Mediterranean countries. Spain carries out trachoma control through a network of clinics helped by the World Health Organization and the United Nations International Children's Emergency Fund; in Greece, treatment and control are carried out through a chain of anti-trachoma dispensaries. In Italy provincial anti-trachoma associations have been set up in co-operation with public and school dispensaries, specialized hospital services, and district health visitor services. Turkey is gaining control over the disease through an increase in the number of dispensaries. Yugoslavia has made a successful attack on its foci of trachoma: senior medical students have carried out surveys among people in affected areas, and suspects are called for examination by specialists. Typhus, formerly widespread in eastern Europe, is now under control, and in Romania success has been achieved through co-operation between Government, the sanitation and epidemiological services, and the public. Public instruction in control methods has been given by trade unions, co-operative services and the Red Cross organizations.

An important development in the control of communicable disease has been the recognition of the part that veterinary science can play against zoonoses. In Italy, for example, there is a Veterinary Division within the Ministry of Health, which deals with such diseases as brucellosis, canine rabies and glanders. In Hungary, the *sanepids* (sanitary and epidemiological stations) have established programmes for brucellosis control in agricultural undertakings, helminthiasis control among schoolchildren, and ancylostomiasis control among miners. In Spain close co-operation is maintained between the health authorities and the veterinary services in the control of brucellosis, tuberculosis transmitted by animals, and other zoonoses. Laboratory facilities, an essential element in the control of communicable diseases, are provided by the local *sanepids* in most eastern European countries, at the health offices in the Federal Republic of Germany and at the universities and hospitals in Austria. In England and Wales there is a national network of laboratories, operated on behalf of the Ministry of Health.

In Yugoslavia, the institutes of hygiene undertake special research projects, as well as routine examinations of public health specimens. This arrangement has been particularly helpful in special field campaigns against endemic syphilis and trachoma.

Other preventive services described in the monograph include food and drug control, maternal and child health, school health, occupational health, mental health and alcoholism, care of the aged, dental care, and health education.

ANTARCTIC AERONOMY AND ICE SHEETS

DURING the past ten years the amount of scientific data available from Antarctica has increased by an order of magnitude. The position has at last been reached when it is stimulating and useful to summarize knowledge in many disciplines in the form of maps. The United States, the U.S.S.R. and the United Kingdom all have projects in hand to publish scientific atlases of Antarctic Research.

The Antarctic Map Folio Series is being published by the American Geographical Society under a contract with the National Science Foundation. The price is most reasonable and indicates that considerable and well-justified financial support has been given by the National Science Foundation. The form of publication and the contents have been based on replies to lengthy questionnaires sent to many interested scientists. The series will consist of about twenty folios, each devoted to one subject or scientific discipline. Each folio consists of a series of maps or diagrams printed on one side of sheets usually 17 × 22 in. and several pages of text of 17 × 11 in.; some plates are also of this size. Each folio series is contained in a light cardboard folder. Four colours are used in the production of maps.

The first folio* deals with aeronomy and has been produced by members of the AVCO Corporation, Wilmington, Massachusetts. A brief text explains the purpose of the observations and the method of compilation. It also includes brief outlines and maps of some data relevant to conjugate points and to the southern auroral zone. The definition of magnetic B and magnetic L values which is given is somewhat involved for the layman. The plates all show ionospheric conditions for 1957 or 1958, the period of maximum sunspot activity. The maps on a scale of about 1 : 100 million show the following parameters: f_0E and f_0F_1 at 0000 and 1200 U.T.; f_0F_2 at 3 h (U.T.) intervals for winter, equinoctial and summer conditions, the percentage of time that $f_0E_s > 5$ Mc/s, and magnetic B and L values at 0, 100, 200, 500, and 1,000 km. The folio should be useful for general reference purposes for scientists in contact with ionospheric problems, but a non-specialist seeking a general appreciation of this work would learn more from a general review article.

The second folio† on the physical characteristics of the Antarctic ice sheet has been compiled in university and other institutes. It deals with several topics of general geographical interest. The Antarctic maps at 1 : 13·6 million are at a scale suitable to the detailed knowledge of many regions. Use of four-colour printing brings out general distributions clearly. Where both colour shading and data figures are shown, as for net annual accumulation of snow and for mean annual temperatures, the effect is good, as one can see clearly the actual measurements and the degree of interpolation. However, the surface elevation and ice thickness maps show neither profile

* American Geographical Society. Antarctic Map Folio Series. Folio 1: *Aeronomical Maps for the Antarctic*. By R. Penndorf, T. M. Noel, G. F. Rourke, and M. A. Shea. Pp. 6+9 plates. (New York: American Geographical Society, 1964.) 3 dollars.

† Folio 2: *Physical Characteristics of the Antarctic Ice Sheet*. By C. R. Bentley, R. L. Cameron, C. Bull, K. Kojima and A. J. Gow. Pp. 10+10 plates. (New York: American Geographical Society, 1964.) 4 dollars.

lines nor points of observation, since the former are shown on a separate map. In theory one superimposes the maps on a light table as necessary—in practice a light table is not always available, and even if it is, the maps differ in placing in relation to the sheet edges, so that careful positioning is needed.

The maps give a reasonably full cover of material available in late 1963, but there are some omissions. Treatment of the Antarctic Peninsula by different compilers is inconsistent, since it is not always considered as part of the main ice sheet. Glaciological data from this region are both very variable and somewhat sparse, but ample information which is available on surface elevations has not been included. The text also states that meteorological observations have been obtained for one year or longer at twenty-one stations on the Antarctic Peninsula or off-lying islands, but the relevant map shows mean annual temperatures for only twelve of these stations. Reliable data for some of the missing stations are available from references given in the useful list of data sources which accompanies the text.

Another obvious omission is that the ice-thickness map does not show data from the French traverse of 1957–58 although the relevant reference is included with the text. Studies of borehole data on temperatures, densities, crystal size and orientation, borehole closure and of stratigraphic profiles are shown diagrammatically on three data sheets. The introductory text is clear, and there is an excellent table setting out various over-snow traverses on the main continent, although the Antarctic Peninsula is again omitted.

For 7 dollars (3 dollars for Folio 1 and 4 dollars for Folio 2), the material supplied is good value and will provide a suitable reference source. Being in separate sheets, the maps can be revised and re-issued as necessary, and even in less than two years since they went to press, and under six months since their arrival in the United Kingdom, substantial additions to many of the maps could already be made. The intention in the United Kingdom and the U.S.S.R. to publish bound atlases at a definite time, rather than loose-sheet folios, has much to recommend it for convenience of librarians and general users. The maps are perhaps more important in supplying background information in fields related to a specialist's own studies, rather than to supply knowledge in his own field. Whatever the map, the specialist will almost always be aware of later data which are missing.

G. de Q. Robin

OBSERVATIONS OF POLARIZED OH EMISSION

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and
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In a recent article Weaver, Williams, Dieter and Lum[1] reported strong microwave emission lines in the *HII* regions *W3*, *W49*, *W51*, *W75*, *NGC* 6334 and Orion *A* at a frequency of approximately 1,665 Mc/s. In some cases the lines exhibited complex spectral structure, and in all cases the emission at 1,667 Mc/s bore little resemblance in intensity and/or structure to the 1,665 Mc/s emission. In particular, the emission in the region of *W3* was approximately 30° K at 1,665 Mc/s, while no emission was detected at 1,667 Mc/s. Weaver *et al.* concluded that the observations could not be entirely attributed to OH and postulated the existence of an unidentified microwave line which they called "mysterium".

In this article we wish to report observations of emission lines near the source *W3* which reveal that: (*a*) emission is present not only at 1,665 Mc/s but also at 1,667 Mc/s and 1,720 Mc/s with frequency spacings as predicted from the OH molecular spectrum[2]; (*b*) some of the emission at 1,665 Mc/s is linearly polarized by as much as 37 per cent; (*c*) the position of the maximum 1,665 Mc/s emission is displaced from *W3* by approximately 14 min of arc; (*d*) emission features as narrow as 1–1·5 kc/s have been observed. As yet we have not observed other regions. In our opinion, "mysterium" is anomalously excited OH.

The observations were conducted using the 120-ft. parabolic antenna of the Haystack Research Facility[3] of Lincoln Laboratory, Massachusetts Institute of Technology, and a spectral-line autocorrelation radiometer. The radiometer is similar to that used in the initial discovery of the OH lines[4]; however, the present radiometer is an entirely new system with several improvements. A room-temperature parametric amplifier is incorporated in the radiometer, and a digital correlator allows the examination of the spectrum in band-widths which can be selected from 40 kc/s to 4 Mc/s. The frequency resolution is 1/40 of the band-width. A Univac 490 computer is coupled directly to the radiometer output and produces the frequency spectrum immediately after the end of each pre-set integration time. The antenna beam-width is 23 min of arc and the overall system temperature is approximately 200° K. The measured continuum antenna temperatures were approximately 240° K on Cassiopeia *A* and approximately 12° K on *W3*.

Typical emission spectra at 1,665, 1,667 and 1,720 Mc/s are shown in Fig. 1 and the results of our observations are summarized in Table 1. Because of our ability to examine the spectra with a frequency resolution of 1 kc/s, more lines are reported in Table 1 than reported by Weaver *et al.* Most of our observations were also made at the position of maximum 1,665 Mc/s emission, $\alpha = 2\text{h } 23\text{m } 29\text{s}$, $\delta = +61° \; 38{\cdot}1'$ (1950·0), slightly displaced from *W3*. The region of strong emission at 1,665 Mc/s

Table 1. Summary of Principal OH Spectral Features

Feature No.	Velocity (OH) (km/s)	Feature width (kc/s)*	Average antenna temp. (°K)	Polarization (per cent)	Position angle	Comments
1,665 Mc/s, 1 kc/s resolution:						
1	−49·1	1·3 ± 0·3	6·1 ± 0·6	30 ± 12	145° ± 10°	
2	−46·5	3·0 ± 0·4	14·3 ± 1·5	2 ± 6		Appears as two or more blended lines
3	−45·2	3·0 ± 0·2	35·4 ± 3·0	37 ± 6	65° ± 4°	Line profile changes with feed position angle
4	−44·5	1·7 ± 0·3	7·0 ± 0·7	22 ± 16	107° ± 22°	Line blended with adjacent lines
5	−43·7	1·6 ± 0·2	14·8 ± 1·5	22 ± 10	140° ± 16°	
6	−43·1	1·2 ± 0·4	3·1 ± 0·3	10 ± 10		
7	−41·7	1·6 ± 0·3	7·2 ± 0·7	16 ± 16		
1,667 Mc/s, 1 kc/s resolution:						
1	−45·5	3·0 ± 0·5	2·2 ± 0·3	10 ± 10		
2	−43·8	1·2 ± 0·5	1·3 ± 0·3			
3	−43·0	1·6 ± 0·5	1·7 ± 0·3			
1,720 Mc/s, 3 kc/s resolution:						
	~ −44		0·85 ± 0·3			Spectrum not resolved due to poor signal-to-noise ratio

* Feature widths given have instrumental width removed.

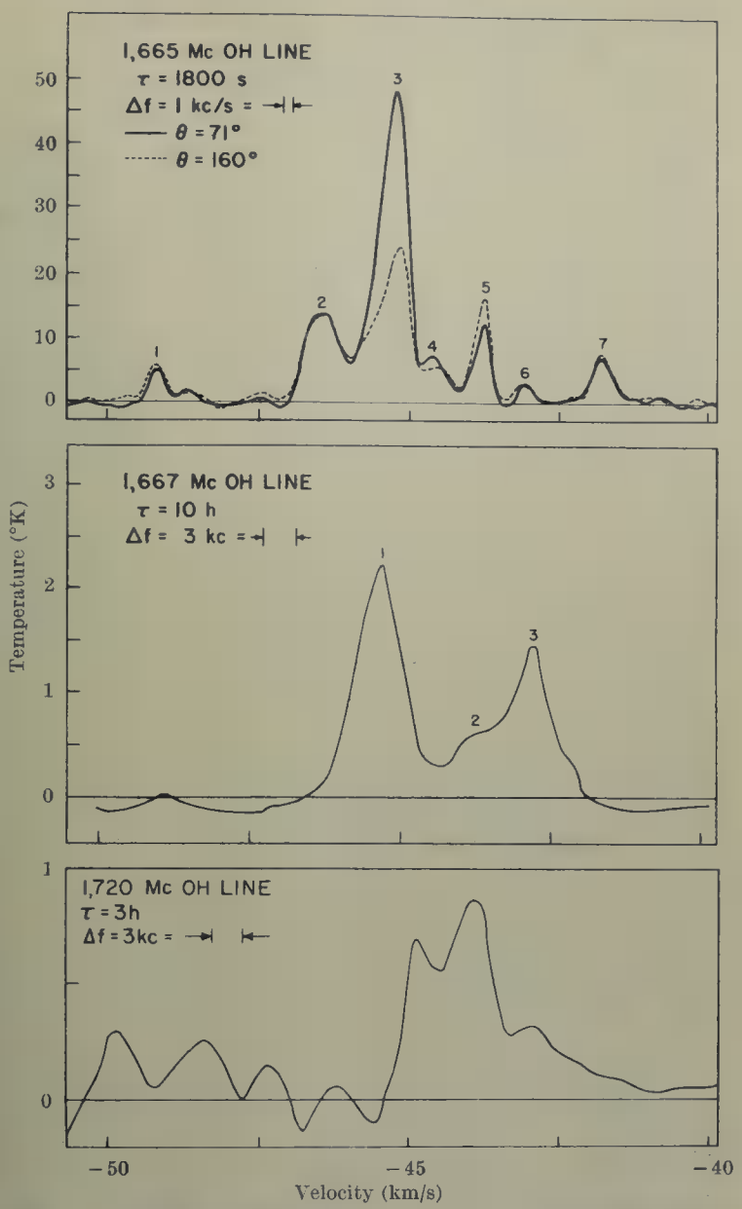

Fig. 1. Examples of OH-line spectra at 1,665, 1,667 and 1,720 Mc/s. The antenna temperatures are shown as a function of velocity relative to the local standard of rest. The data presented in Table 1 are, in some cases, of longer integration time and higher resolution than the examples presented in this figure

appears to be of slightly greater angular extent than our beam-width, as adjudged by the intensity of the −45 km/s line. The values of Table 1 must be considered uncertain to the extent that blending of the lines occurs in some cases. Spectra with 1 kc/s resolution at various polarization angles reveal that some of the lines may be quite complex and consist of two or more lines blended together. The observations also suggest that a small, but significant, amount of absorption may be present, particularly at 1,667 Mc/s, thereby making interpretation of the spectra difficult without further observations.

The observations clearly show that a large anomaly exists in the intensity ratios of the lines. For small optical depths, the intensities for the 1,665, 1,667 and 1,720 lines have theoretical ratios of 5 : 9 : 1 whereas our observations give approximately 50 : 2 : 1. The 1,612 Mc/s line was not detected during our limited search and its intensity appears to be less than the 1,720 Mc/s line. We observe a maximum antenna temperature of 50° K for the 1,665 Mc/s emission which implies, from considerations of antenna efficiency and beam filling, that the brightness temperature is in excess of 200° K. On the other hand, the narrowness of the lines implies maximum kinetic temperatures of 15°–30° K. Therefore, the population distribution among the OH energy-levels must be established to a large degree by radiative processes and collisions are relatively ineffective.

The existence of strongly polarized OH emission is both surprising and difficult to explain. Possible polarization mechanisms are: (1) Zeeman effect; (2) Stark effect; (3) resonance scattering; (4) amplification, through a maser-type population inversion, of polarized background radiation. The approximate difference of 90° between the position angles of the −45·2 km/s line and the −43·7 and −49·1 km/s is characteristic of the Zeeman effect when viewed normal to the magnetic field. If the polarization is due to the Zeeman effect, magnetic fields of the order of 5×10^{-3} gauss are indicated. The Zeeman effect is unique among the four mechanisms listed above because it is the only mechanism which will give rise to circular polarization. Therefore, observations of this region with circular polarization are extremely important. Furthermore, if magnetic fields of the order of milligauss are present, 21-cm emission and absorption in the neighbourhood of *W*3 should be critically examined.

We thank G. H. Conant, jun., and P. P. Crowther and other contributions by the staff of the Haystack Research Facility for their assistance.

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[1] Weaver, H., Williams, D. R. W., Dieter, N. H., and Lum, W. T., *Nature*, **208**, 29 (1965).
[2] Radford, H. E., *Phys. Rev. Letters*, **13**, 534 (1952).
[3] Weiss, H. G., *I.E.E.E. Spectrum*, **2**, 50 (1965).
[4] Weinreb, S., Barrett, A. H., Meeks, M. L., and Henry, J. C., *Nature*, **200**, 829 (1963).

OH EMISSION IN THE DIRECTION OF RADIO SOURCE *W*49

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FOLLOWING the first detection of microwave lines of OH in emission near the source *W*49 by Gundermann[1] and by Goldstein and Gundermann[2] at Harvard University, similar emission was observed in a number of other galactic radio sources[3]. These initial investigations revealed a number of surprising results, particularly that the $F = (1 \rightarrow 1)$ 1,665 Mc/s transition has a greater intensity than the $F = (2 \rightarrow 2)$ 1,667 Mc/s transition, a reversal of the expected result.

In this article, we discuss recent detailed observations of OH emission in the direction of the source *W*49 and preliminary results for *W*51. We have obtained 3 kc/s resolution spectra of *W*49 at the OH frequencies of 1,665 Mc/s and 1,667 Mc/s with two radio telescopes: the Harvard University 60-ft. radio telescope and the 120-ft. Haystack telescope of the Lincoln Laboratory of the Massachusetts Institute of Technology[4]. These spectra have been quantitatively compared with each other. We have also obtained 1 kc/s resolution spectra at 1,665 and 1,667 Mc/s using the Haystack antenna. In view of the discovery of polarization of OH radiation in the source *W*3 by Weinreb, Meeks, Carter, Barrett and Rogers[5], the *W*49 spectra have been checked for polarization and also for possible time variations. OH spectra in the region surrounding *W*49 have been obtained in an attempt to determine the angular size of the OH emitting

region and its position relative to the continuum source *W49*. Finally, using the results obtained on *W49* and *W51*, we have carried out a preliminary investigation of the possibility that a spatial correlation exists between OH observed in emission and associated continuum sources.

The Harvard radio telescope is equipped with a cavity maser radiometer and a multi-channel filter bank composed of 10 units with available band-widths of 3, 25, 80 and 150 kc/s. At the OH frequencies, the antenna has a half-power beam-width of 52 min of arc and its aperture efficiency is 0·38. The mode of operation switches the radiometric system between the antenna input and a resistive comparative load maintained at approximately 4° K by immersion in liquid helium. The switching rate between the comparison load and the antenna is 3·5 c.p.s. An additive comparative noise source balances the system during operation. The total system noise is approximately 100° K. The characteristics of the Haystack OH spectral line system have been discussed by Weinreb *et al.*[5].

A quantitative comparison shows that the 3 kc/s resolution spectra for *W49* obtained with the Harvard and the Haystack telescopes are practically identical in shape and differ only in amplitude. If an OH cloud and a continuum source are observed with two radio telescopes of different apertures, and if the cloud and source are small compared to the beam-widths of both telescopes, then the amplitude of each spectral feature should be proportional to the effective apertures of the telescopes. In this investigation, the ratio of effective apertures (Harvard/Haystack) is $\cong 0{\cdot}38$. Using the total antenna temperatures at Harvard and at Haystack, we found that the observed ratios for the major spectral features at +17 km/s and +21 km/s (1,665 Mc/s transitions) are $\sim 0{\cdot}33$. If we take into account the known differences in effective filter shapes at Harvard and at Haystack, the observed ratio agrees quite well with the ratio of effective apertures.

We made a direct investigation of the angular size of the OH region responsible for the emission features at 1,665 and 1,667 Mc/s, by using 3 kc/s spectral resolution and the Haystack antenna. Comparison spectra were obtained for a number of positions close to *W49*. We were unable to detect any noticeable broadening of the Haystack beam, using the strong features at +17 and +21 km/s. Both the angular probe in position and the scaling of the spectral features imply that the region responsible for the OH emission is small with respect to the 23 min of arc beam-width of the Haystack antenna. The position of maximum OH emission lies at $\alpha = 19\text{h}\ 7\text{m}\ 55\text{s}$ and $\delta = 9°\ 1'$ (1950·0).

Fig. 1 presents the spectrum of *W49* obtained at the Haystack facility with a band-width resolution of 1 kc/s. The total integration time is 15 min. Numerous narrow spectral features are observed ($\sim$2–3 kc/s at half maximum) and there is little correlation between the spectral shapes at 1,665 and 1,667 Mc/s.

The expected total line-width at half-power due to a thermally broadened cloud of OH is:

$$\Delta\nu \text{ (cycles/sec)} = 289\sqrt{T}$$

For the strongest features, the maxima observed in Fig. 1 are approaching the limit permitted by thermal broadening. However, since the OH emitting region is small in angular size, the apparent temperatures will continue to increase when observed by telescopes with larger effective areas and will exceed amplitudes $\sim 10^2$ °K. This argues for radiative excitation of the OH lines in the direction of *W49*, similar to the situation of *W3* as reported by Weinreb *et al.*[5]. The continuum temperature which lies beneath the base-line in the two spectra in Fig. 1 is $T_c = 13° \pm 1°$ K.

In view of the polarization of the radiation from a similar source *W3*, as announced by Weinreb *et al.*[5], we have examined the 1,665 Mc/s peaks at radial velocities of +17 and +21 km/s with 3 kc/s and 1 kc/s band-width resolutions respectively for polarization effects. In each case, we made six measurements of the amplitude of the features, rotating the plane of polarization of the Haystack antenna feed system by approximately 30° between adjacent measurements. Using a definition of the percentage of polarization $p = 100\ (T_1 - T_2)/(T_1 + T_2)$, where T_1 and T_2 are the maximum and minimum temperatures obtained with appropriate orthogonal positions of the antenna feed system, we conclude that the polarization of the features examined has an upper limit of 8 per cent.

We have examined our data for possible time variations in the +17 and +21 km/s features. There is nothing in the data to indicate that such an effect is present.

The prediction by Kardashev[6] and the successful detection of several lines of excited hydrogen with transitions of the $n \rightarrow n-1$ type raise a question of possible confusion for OH spectral line research. Transitions of the $n \rightarrow n-1$ type occur throughout the region of the four OH lines when n takes on values between 155 and 160. However, calculations show that none of these hydrogen lines falls sufficiently close to the four OH lines to contaminate OH spectral analysis, and of the six lines that bracket the OH frequencies, none falls within 10 Mc/s of the OH lines.

The continuum source *W49* has been studied by a number of workers. Bechis and Penfield[7], utilizing the Haystack antenna at 8·25 Gc/s, have obtained a radio map of the region *W49* clearly showing the two principal components of the source. Both components have angular widths of 3–4 min of arc and are separated by about 13 min of arc in right ascension. Data obtained at Green Bank[8] at 750 Mc/s and at 1,400 Mc/s and at Haystack (8 Gc/s) suggest that one source is thermal and the other non-thermal (spectral index $\cong -0{\cdot}6$), their flux densities being equal at approximately 750 Mc/s. Weaver *et al.*[3] noted that wherever OH had been found in emission (six sources) there was an HII region somewhere along the line of sight. If there is indeed an actual spatial correlation between continuum sources and OH emitting clouds, as our results on *W49* and *W51* suggest, then we can obtain a distance to the thermal component of *W49* as well as to the clouds responsible for the OH spectra.

Akabane and Kerr[9], utilizing the Parkes antenna, have attempted to deduce the distance to the source *W49* by

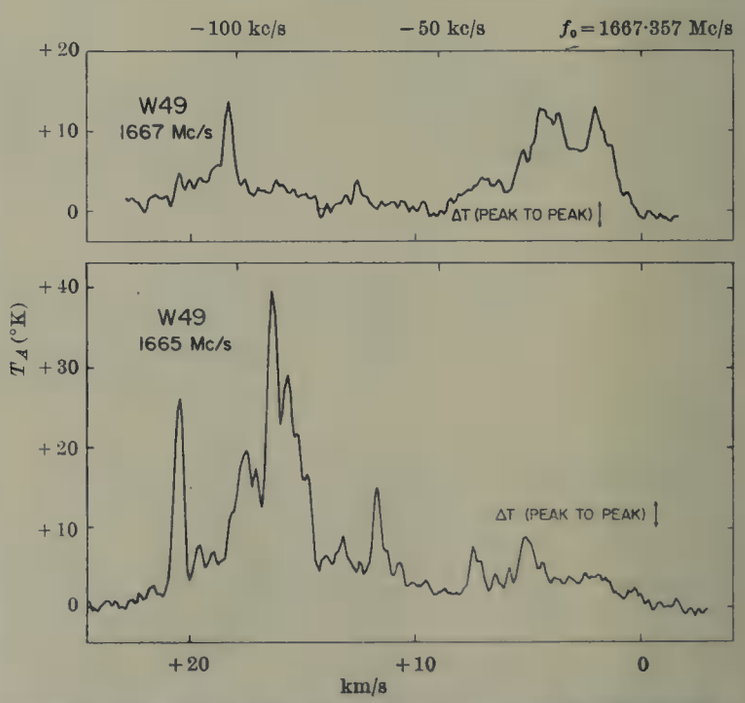

Fig. 1. Spectra of *W49* obtained with a resolution of 1 kc/s and an integration time of 15 min. The abscissae are frequency and velocity with respect to the local standard of rest. The ordinates are antenna temperature. The above spectra were obtained for the direction α = 19h 7m 55s, δ = 9° 0′ (1950)

examining the absorption at 21 cm of its continuum radiation due to intervening HI. From their work, it appears that the non-thermal component, located some 10 min of arc away from the galactic plane, was essentially out of their beam as they observed the thermal source on the galactic equator. For a Sun-centre distance of 10 kpc, they arrive at a probable distance of 15 kpc and a possible alternative distance of 7·5 kpc.

Utilizing the standard differential theory of galactic rotation, we have inferred that the OH clouds are approximately either 14 kpc or 1 kpc distant from the Sun. If we assume that the OH clouds are physically close to the HII region, then this unambiguously places the OH clouds and the thermal component of *W*49 at about 14 ± 1 kpc. We found that the position of the greatest line amplitude of the major features at 1,665 Mc/s and 1,667 Mc/s was within ±1 min of arc of the thermal component of *W*49 and unrelated to the non-thermal component. This argues in favour of a close physical association of the OH clouds and the thermal source. With a close physical association, the thermal region could be the radiative source responsible for the OH line excitation.

A further argument for associating the OH and continuum sources arises from our 8·25 Gc/s studies at Haystack of the source *W*51, which also appears to be complex, consisting of four components. Although we have not yet had an opportunity to examine this region in detail, we find, with Hoglund and Mezger[10], that the source at α (1950) = 19h 21m 20s, δ (1950) = 14° 23′ (component *A*) is the strongest one at 8·25 Gc/s. We have noted OH emission at 1,665 Mc/s in *W*51, using 3 kc/s resolution at Haystack. We easily distinguished between the Westerhout position and the component *A* position, their separation being 17 min of arc. At the Westerhout position, we found an OH signal which, if real, was barely above the noise, whereas at the position of component A we noted a number of emission features; the strongest was about 10° K.

Additional indication that a spatial correlation may exist between the OH in emission and continuum sources comes from a study of radial velocities in *W*51. The OH velocity was +58 km/s, whereas from a study of two newly discovered lines of excited hydrogen[11] in this source, we found ~60 km/s for a thermal source located in *W*51. The observed thermal region and the OH clouds appear to lie at the point of maximum radial velocity along the line of sight in their direction in l^{II}, b^{II}, which suggests that their distance is about 6·5 kpc.

Wherever OH has been found in emission there appears to be an associated HII region. Therefore, it appears profitable to search for excited hydrogen lines in areas where OH is discovered in emission.

We thank M. L. Meeks, S. Weinreb, A. E. E. Rogers, and J. C. Carter for their assistance. The programme in radio astronomy at Harvard is supported by the National Science Foundation. Lincoln Laboratory is a centre for research and development operated by the Massachusetts Institute of Technology with support of the U.S. Air Force.

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COMPARISON OF EXPERIMENTAL DATA OF THE EFFECTIVE ION MASS IN THE UPPER IONOSPHERE WITH A THEORETICAL STRUCTURE

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ELECTRON-DENSITY profiles obtained from evaluation of the readouts from the U.S.–Canadian topside-sounder satellite *Alouette* permit the measurement of the scale height in the upper ionosphere, from which information can be obtained[1–4] regarding the composition of the ionospheric regions. We centred our evaluation of the topside ionograms on the task of finding directly[5,6] the exponential functions that govern the electron-density profiles. Thus, by the same process of numerical approximation, we obtain the electron density and the scale height. Therefore the computation reflects the main natural irregularities that are not smoothed out as by a differentiation of a density profile.

Our computation describes the electron density versus altitude profile in terms of an exponent α and the altitude h as follows:

$$N(h) = N_0 \exp(-(h-h_0)\alpha) \qquad (1)$$

where N and N_0 are the electron densities at altitude h and h_0 respectively; the exponent α is a function of h. Under the assumptions mentioned below the exponent α can be considered the inverse electron-scale height $1/H$. Changes from region to region (of different but constant α) are made by our computation programme when the α for best data fit of a specific region has to be changed by more than the arbitrarily set 3 per cent. This setting results in an eventual uncertainty of the scale height by not more than 6–9 km within the altitude range we are going to consider.

Typical scale height versus altitude curves at daytime are given in Figs. 1–4. It is interesting to note a special fine-structure in the scale-height profiles. Very often data are presented in groups of constant exponent, thus reflecting a laminated structure (first observed by King[1]). Each electron-density profile contains an inflexion point

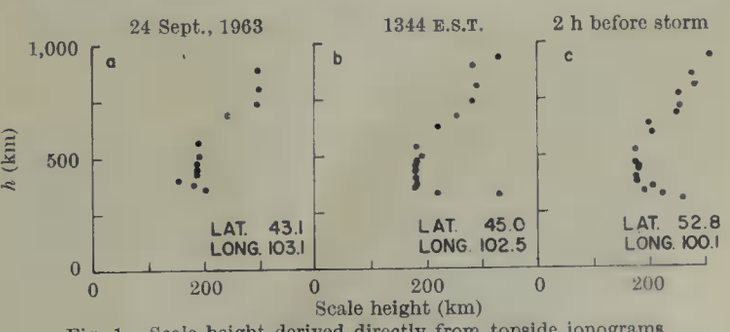

Fig. 1. Scale height derived directly from topside ionograms

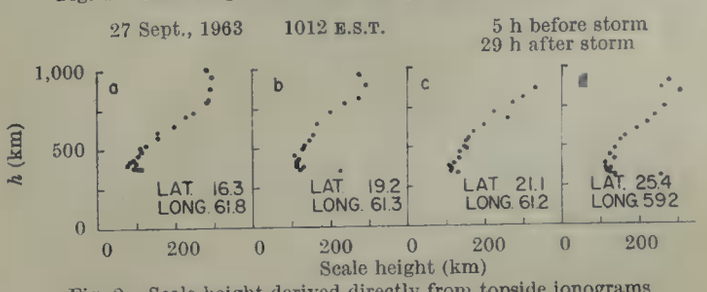

Fig. 2. Scale height derived directly from topside ionograms

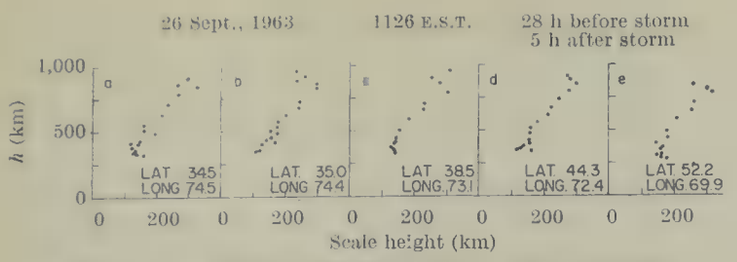

Fig. 3. Scale height derived directly from topside ionograms

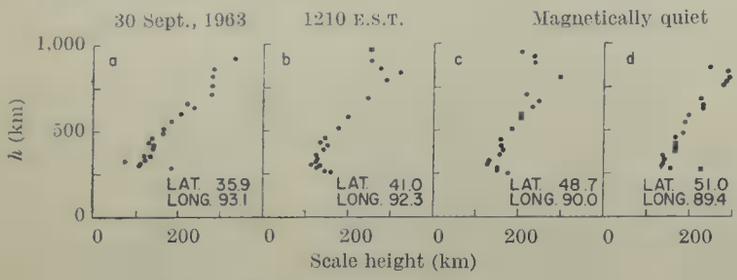

Fig. 4. Scale height derived directly from topside ionograms

where the curvature from the concave bending around f_0F_2 maximum changes to the convex curvature of exponential decay in the higher ionosphere. Obviously, a constant slope (that is, a constant exponent) will always be observed at the altitude of this inflexion. The altitude of inflexion is necessarily a function of the overall shape of the profile and might thus be related to seasonal, geographic, and other parameters. It appears to be rather pronounced at the magnetic equator[1]. However, it is questionable whether a specific physical interpretation can be correlated to this inflexion point.

Our investigations[6] of the altitude changes of typical plasma frequencies during magnetic activity have shown that the onset of a storm might be accompanied by a decrease in electron density. Such decreases may continue longer than the storm lasted as indicated by the local K index. It is plausible, therefore, that the slope of the scale-height profile is affected by magnetic events. The data shown in Fig. 1 (taken 2 h before a storm) and in Fig. 3 (taken 5 h after a storm) represent such magnetically disturbed conditions. A typical set of undisturbed data appears to be Fig. 4, which was taken more than a day after the last storm and more than ten days before the next storm. Comparison of Figs. 3 and 4 indicates that immediately after a storm the slope of the scale height is increased. During magnetically quiet periods (Fig. 4) the plateaux of constant exponent are repeated at nearly the same altitude during the passage of the satellite at the various points along its trajectory (36°–51° N.). As indicated in Fig. 4 and Table 1, one can distinguish, during this passage for example, about five groups or plateaux, each of which is represented by a specific constant value. An average over the above latitude range taken during the same satellite passage results in five data pairs that can be considered representative for this latitude region at this specific time. Fig. 5 gives the end values from Table 1 in graphical form. The rectangles in Fig. 5 represent the spread of the single data. The lower three rectangles cover the entire range of the pertinent scale, indicating a really laminated structure that is less pronounced at higher altitudes partly because fewer data points are obtained there by our evaluation procedure. The combination of simultaneous data from various latitudes appears permissible in view of the fact

Table 1. Averaged Scale Height
September 30, 1963. 1200 E.S.T.

Lat.	Long.	Group 1 H	Group 1 h	Group 2 H	Group 2 h	Group 3 H	Group 3 h	Group 4 H	Group 4 h	Group 5 H	Group 5 h
35·9	93·1	283	784	221	631	171	521	138	442	125	347
41·0	92·3	329	823	244	683	189	546	143	423	124	340
48·7	90·0	297	800	231	697	197	550	157	407	129	313
51·0	89·3	284	783	227	650	188	536	166	412	137	325
Averages		298	798	231	665	189	536	151	421	129	332

H, scale height; h, real height, both in km.

that the ionosphere above our region has been found very well stratified[6] during magnetically quiet periods. The observed narrow spread of the data encourages the use of these data for a typical model (daytime, mid-latitude).

Considering the portions of the electron-density profile where constant exponents are observed, one might conclude that within such a range electrons and ions are each in specific isothermal equilibrium. In this case the inverse exponent is equivalent to the electron-scale height H:

$$\frac{1}{\alpha} = H = R(T_i + T_e)/Mg \qquad (2)$$

Here R is the universal gas constant, T_i and T_e are the kinetic ion and electron temperatures, g is the acceleration of gravity and M is the effective ion mass (in atomic units); all parameters are a function of height; however, $T_i + T_e$ are considered constant for each region of constant $1/\alpha = H$. Since H and g are known, equation (2) reveals[4] the ratio $(T_i + T_e)/M$ as a function of laminated altitude. The variation with altitude of equation (2) can be described by:

$$\frac{1}{M}\frac{dM}{dh} = \frac{1}{T_i + T_e}\frac{dT_i}{dh} - \frac{1}{H}\frac{dH}{dh} - \frac{1}{g}\frac{dg}{dh} \qquad (3)$$

when it can be assumed that T_e is close to a constant value[7,8] in the altitude range under consideration (300–1,000 km). The function $T_i(h)$ is not known exactly. Therefore, an empirical solution is suggested. A comparison of the possible magnitude of $\triangle M/M$ and $\triangle T_i/(T_i + T_e)$ as it can be obtained from the literature[8,9] indicates that, for the altitude range in question, one might assume:

$$(1 \pm \varepsilon)\frac{\triangle M}{M} = -\frac{\triangle T_i}{T_i + T_e} \qquad (4)$$

where ε is a correction term which is also a function of altitude. The used numerical comparison of the terms in equation (4) is based on the neutral particle composition given by Harris and Priester[9] for the mass data. This composition is not considered identical with the ion mass composition; however, the relative change in magnitude is assumed similar for neutrals and ions. A justification for equation (4) might be seen in the possibility that the lighter ions are more easily heated than the heavier ones by their interactions with the electrons. This effect will become more pronounced at higher altitudes.

Application of the empirical equation (4) permits a solution of (3) in case an assumption is made for the magnitude of $\varepsilon(h)$. If we can assume $\varepsilon = 0$ for the altitude interval in question we arrive at:

$$\left|\frac{dM}{dh}\right| = \frac{M}{2}\left\{\frac{1}{H}\left|\frac{dH}{dh}\right| - \frac{1}{g}\left|\frac{dg}{dh}\right|\right\} \qquad (5)$$

For $\varepsilon \neq 0$ the above denominator reads $2 \pm \varepsilon$ instead of 2.

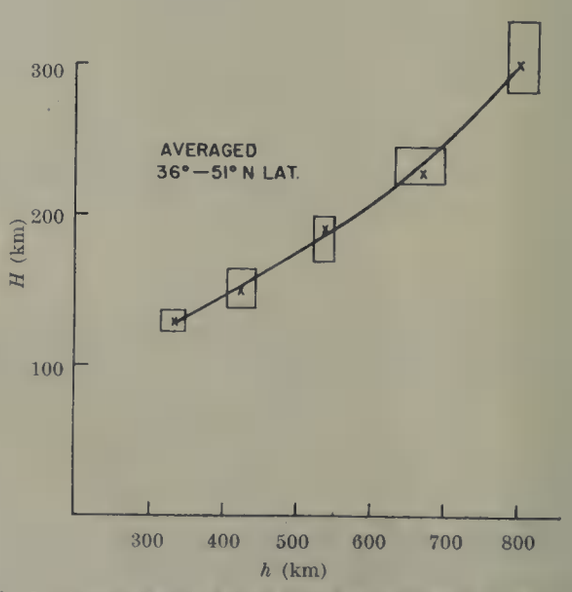

Fig. 5. Averaged scale height during magnetically quiet period

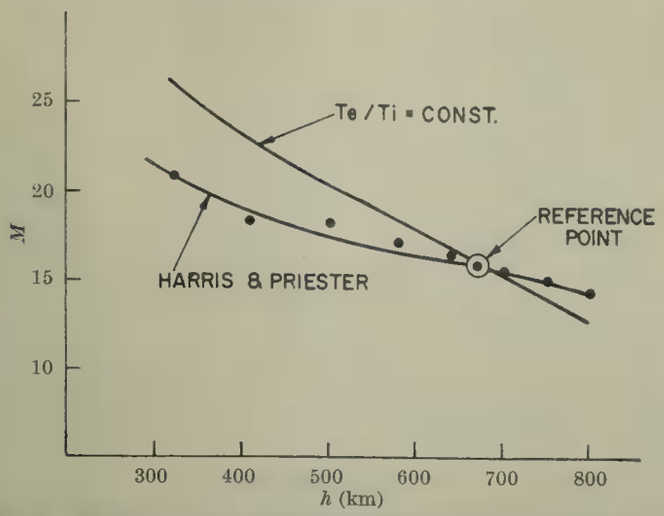

Fig. 6. Effective ion mass versus real height. Lower curve, Harris and Priester data for neutral atmosphere; upper curve, data from Fig. 5 converted for isothermal equilibrium of electrons and ions. Dots: data from Fig. 5 converted for non-isothermal conditions as indicated by equation (5)

Equation (5) can be numerically solved, for example, by insertion of data from Table 1 provided, however, that a reasonable value is obtained for an initial reference point M, h. For this reference we have chosen rather arbitrarily the value of the altitude that Harris and Priester ascribe to $M = 16$ for daytime (11 h L.M.T.) under consideration. This value is 670 km, to which we co-ordinate the experimental value of $H = 245$ km from Fig. 5. With these assumptions we obtain the data points for M as shown in Fig. 6. For comparison the original curve from Harris and Priester is also shown in Fig. 6 as well as another evaluation of our scale-height data which was based on the assumption that both T_i and T_e were constant. Obviously, both evaluations are not correct. The upper curve deviates apparently too much towards higher M values. The good correlation of the curve described by the row of points in Fig. 6 with the Harris and Priester curve cannot be taken as finite establishment of the applied correction term $\varepsilon = 0$. However, it is taken as an indication that an application of equation (5) comes closer to the true composition than the assumption of a completely isothermal medium (T_i and T_e/T_i = constant).

Bauer has informed me that he has referred to a more realistic reference point derived from rocket probes for the ionic mass 16, which point is related to an average altitude of 300 km, that is near the inflexion point of the profile. Thus the dotted curve in Fig. 6 has to be moved downwards to show a variation of M between 16 and 7 in the range 300–800 km.

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DEPENDENCE OF THE PROPERTIES OF MOVING STRIATIONS IN THE PLASMA OF THE POSITIVE COLUMN ON SOME EXTERNAL AND INTERNAL PARAMETERS

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In general, gas and vapour discharges occur associated with a number of oscillatory phenomena. Even under the simplest conditions, in the event of direct current discharges, a number of discharge anisotropies or instabilities in the state of equilibrium of the plasma can be demonstrated, which may lead to the generation of oscillations. Of these oscillations there is one which stands out because of its regularity. This is the so-called plasma oscillation which has been demonstrated and theoretically discussed by Langmuir and Tonks[1]. These oscillations are characteristic of the given discharge plasmas; they accompany them all, and may be demonstrated in the positive column by means of a pair of Lecher-wires. Their frequency can be written:

$$n_e = \left(\frac{N_e \cdot e^2}{\pi m_e}\right)^{1/2} \qquad (1)$$

where n_e is the frequency, N_e is the electron concentration, e the charge of electron, and m_e the electron mass.

The same relationship holds good for ions merely substituting the corresponding numerical values in the formula. For electron oscillations, using equation (1), a value of about 10^8 c/s is obtained for the frequency at an electron concentration of 10^{10} electrons/c.c. The frequency for ion oscillations, however, is much lower, due to the mass proportion and concentration assumed being smaller in certain instances.

In addition to these typical plasma oscillations (electron and ion oscillations), there is still another oscillation process worth mentioning—a process which also regularly accompanies direct current discharges. This process is known as 'striation', and two specific forms of the process can be distinguished[2], namely the standing and moving striations.

As for the frequency, the study of striation does not require the rather complex techniques required for the so-called plasma oscillations, and for striations the frequency can be shown to be in the 10^2–10^4-c/s range. Results published in the literature in this field have already been discussed by one of us in an earlier paper[2].

A number of research workers are at present engaged on investigations of these striations, and are exploring their generation under various conditions. So far as the origins of the striations are concerned, no uniformly accepted theory has as yet been formulated. Certain research workers are investigating the processes occurring in the region of the electrodes for the anisotropy responsible for the phenomenon of striation—which can be demonstrated in the positive column and which is generally accompanied by variations in light. Others believe the origin of striation to be in the temporal changes of the basic processes of the positive column (excitation, diffusion, variation in the concentration of metastable atoms, recombination, ionization, etc.). Some are concerned with investigations of parameters influencing these striations[2–4], and by way of these studies are trying to draw conclusions as to their physical nature. Using the earlier findings of Szigeti[8], one of us[9] has succeeded in demonstrating the influences of certain internal plate-side parameters of the discharge tube on the moving striations within the positive column.

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of a low-pressure gas discharge. Studies are also being undertaken on influences external to the gas discharge tube which affect the moving striations. According to Yoshimoto *et al.*[10] the velocity of the moving striations can be influenced by certain external conditions (ambient temperature, external resistance, etc.) in a critical manner. Pekarek[11] also mentions a weak influence of external circuits on the moving striations. Moreover, according to Nedospasov *et al.*[12], the external effects will, in addition to the velocity, influence the wave-length of the striations.

Donahue[13] believes that for constant electrical and external parameters (temperature, geometry, etc.) the phenomenon of striation characteristic of direct current discharges can be considered as a superposition. In this case the moving striation is characterized by two space-charge waves moving in directions opposed to each other. In the discharge tube these space-charge waves move in conformity with the sign of the space-charge in opposing directions. Of these waves the negative space-charge wave composed mainly of electrons is characterized by a higher velocity.

The subject-matter of the tests to be described here was the investigation of the characteristic properties of these higher-speed space-charge waves at a variety of discharge currents. The instrumentation and set-up of the measurements performed during the tests were similar to those described by Donahue[13]. We have already described this set-up in an earlier paper, which deals with the pressure-dependence of the striation frequency and the speed of propagation[3].

Striation in the direction of the axis of the positive column may be controlled by factors inside and outside the discharge in a number of ways[2,4,5]. Thus, among other effects in the event of using Penning gas (that is, a gaseous mixture of mercury and argon), the process of striation is likely to be influenced by variations in the ambient temperature, through the variation of mercury–vapour pressure and the consequent variation of the concentration of the neutral atoms. It is a well-established fact that in the event of mercury vapour discharges the concentration of mercury atoms in the discharge is determined by the wall temperature of the discharge vessel through the partial pressure resulting from the saturated vapour pressure of the mercury.

Another object of the tests reported here was to study, using a Penning gas, the influence of certain external and internal parameters on the moving striations of the d.c. mercury vapour discharge, and to explain the dependence of the striations on these parameters. The following were included: as external parameters, the limiting resistance and ambient temperature; as internal parameters, the discharge curve.

No investigations of the external parameters of moving striations are known to us from the literature which have included studies of the dependence of temperature. Such tests would be justified by the aforementioned character of the mercury-vapour discharge in this type of d.c. discharge. For the theoretical description of the phenomenon of oscillation we accepted the calculations made by Kapzow[15] for an equivalent circuitry, and followed the trend of thought of his calculations.

For purposes of measurement, we made use of the method introduced by Donahue[13] and described by us in detail in an earlier paper[3]. Accordingly, in order to test the frequency of a moving striation, its nodes and light maxima, recourse was had to a method using photocells, and, by passing the signals of the photocell through an amplifier-stage, direct observations could be made on an oscilloscope screen. With the use of the oscilloscope the wave-length and frequency could be estimated (by means of Lissajoux curves). Furthermore, the light intensity of the striae formed during striation could be investigated along the discharge tube as a function of distance.

The discharge was supplied by a stabilized d.c. source while the discharge current was limited by symmetrically arranged ohmic resistances in series. The discharge tube used on this occasion had a glass wall 1 mm thick; its length was 1,200 mm, and its outer diameter was 38 mm. After the appropriate vacuum treatment the tube was filled up with a mercury–argon gas mixture at a mercury column pressure of 3 mm, and about 60 mg mercury. To maintain the mercury vapour pressure at a constant value the discharge tube was placed in a water-jacket, and water was circulated continuously at a fixed rate. By using an ultra-thermostat, the temperature of the water responsible for temperature stabilization could be set at any value specified with an accuracy of $\pm 0{\cdot}02°$ C. Tests began after a steady-state operating period of the discharge tube of 20 min, at specified predetermined stabilized temperatures of 20°, 25° and 40° C.

(*a*) *Influence of the external resistance on the moving striation.* When these tests were made the discharge tube was supplied from the stabilized d.c. source already mentioned, with an arc current of 100 m.amp, without specially heating the cathode. As an external limiting element during the first period of the tests a variable ohmic resistance was used which had negligible inductance. For the various values of the supply voltage (200, 300, 400 V) the external ohmic resistance was set so that during the tests the value of the current invariably remained 100 m.amp. By means of the measuring methods already described, and for a stabilized temperature of $25 \pm 0{\cdot}02°$ C, the results shown in Fig. 1 were obtained. In Fig. 1 the dependence of the various characteristics on the value of the external ohmic resistance applied can be followed for an arc current of 100 m.amp. Curves are plotted for A, the amplitude of the light intensity variation, for v, the travelling speed of the moving striation, and for the resistance-dependence of the wave-length λ of the striation and the frequency ν. Of the two striation waves of different velocity detectable in the discharge, the parameters in the diagram in Fig. 1 apply to that travelling at the higher speed.

In the second set of tests, in addition to the ohmic resistance in series, an inductance coil, also in series, was used as an external influence. This resistance was alternately inserted or removed, as required. Its value was 1·5 henries, and the ohmic resistance was 10 ohms. With the inductance coil inserted in order to maintain the resultant ohmic resistance of the outer circuit on a constant value, the value of the original variable resistance had been reduced by 10 ohms. The effect of the inductance on the frequency of the moving striation for an arc current of 20 m.amp is shown in Table 1.

Table 1

	Inductance coil	
	Inserted	Removed
Frequency (sec^{-1})	541	498

From Fig. 1 it can be seen that for an unchanged arc current the value of the external limiting ohmic resistance

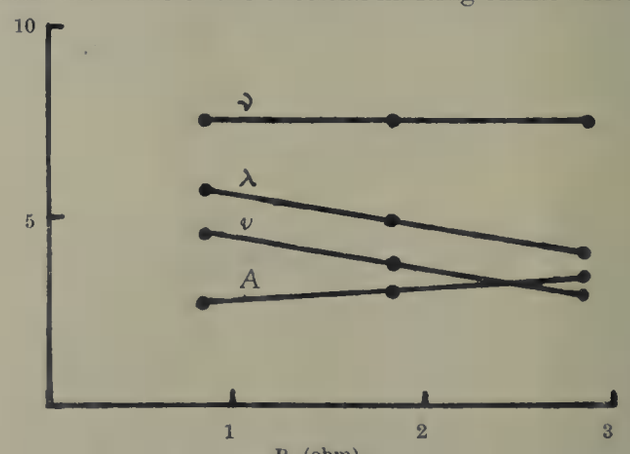

Fig. 1. The various parameters of moving striations as functions of the value of the applied external ohmic resistance for an arc current of 100 m.amp. A, Amplitude of the variation of light intensity in arbit units; V, travelling speed of the moving striation in 10^3 cm sec^{-1}; λ, wave-length in cm; v, frequency in 10^3 c/s units

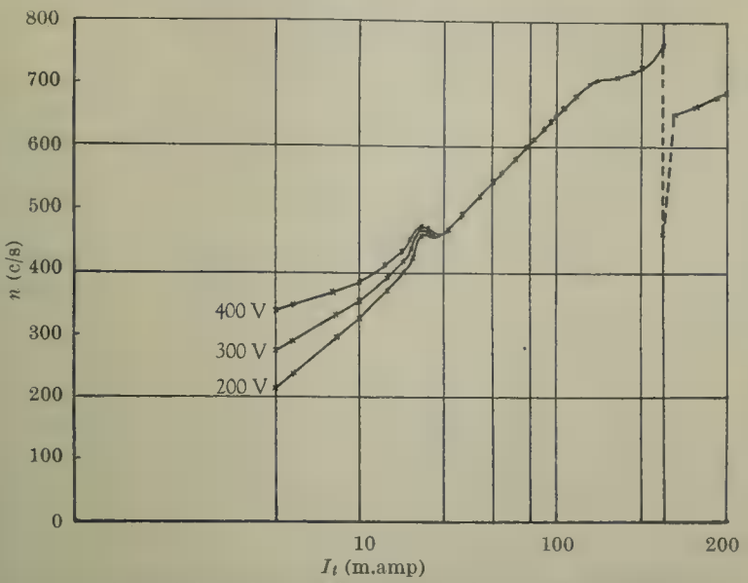

Fig. 2. Current dependence of frequency n of the moving striation for various supply voltages, 200, 300, 400 V; I_t, discharge current

will, in the range under test, influence the amplitude of the light variations in the moving striation, the speed of propagation and the wave-length of the moving striation; however, it will not affect its frequency. The conclusion may thus be drawn that in the range in question the internal parameters of the discharge will have a decisive effect on the frequency of the moving striation.

On the other hand, what Table 1 shows is, provided that the required conditions exist, the presence of the inductive member of the external limiting element affects the frequency of the moving striation, that is, in this case it is not the internal parameters of the discharge alone that determine the frequency.

(*b*) *Influence of the shape of the voltage versus current plot on the moving striation.* In this set of tests, by increasing the range of current, measurements were carried out in the discharge current range between 5 and 200 m.amp.

In Fig. 2 curves have been plotted for the current-dependence of the frequency of the moving striation at a stabilized temperature of 25° C, first in the current range of 20–200 m.amp, and then of 5–20 m.amp. As may be observed, the shapes of the two frequency characteristics differed fundamentally from one another in the two ranges. In the 20–200 m.amp range the frequency of the moving striation could be controlled mainly by means of the internal parameters of the discharge, whereas in the 5–20 m.amp range together with the internal parameters the external parameters were having a remarkable influence on the character of the current-dependency of the frequency.

From the results plotted in Fig. 2, and using the results of measurements made at 20° and 40° C, an empirical formula was derived for the current-dependence of a frequency n in the range between 20 and 70 m.amp:

$$n = n_c(T) + C(T) \log i_t/i_{ot} \qquad (2)$$

where $i_{ot} = 20$ m.amp, i_t denotes the discharge current, n_o is the frequency of the moving striation at i_{ot}, T is the temperature of the thermostating water-jacket, and $C(T)$ is a constant at the given temperature.

It could be shown that with rising temperature T, the value of frequency n_o tended to drop, while the value of C increased.

A further conclusion drawn from the results was that with increasing vapour pressure the frequency was decreasing in the range between 5 and 40 m.amp, whereas it was rising at currents of 120 and 130 m.amp. It should be noted, however, that the curve plotted in Fig. 2 has a rather marked kink at 120 m.amp. The conclusion to be drawn from these results is that the concentration of the mercury atoms, as one of the internal parameters, had a slight effect on the frequency response of the moving striation.

The experimental results established for the current dependence of the light and current oscillations of the moving striation are shown in Figs. 3 and 4. As was observed by Bauer[14] using a supply voltage of 160 V, the light oscillations had a relative maximum when the voltage-current plot had a maximum. With this observation in mind we investigated the problem of the current dependence of the light oscillations, the results of which are plotted in Fig. 3. The amplitude of the light variation, L_o, was a maximum at 50 m.amp, and measurements showed that this maximum was independent of the value of the supply voltage—a circumstance which seems to indicate that the effect was independent of the external resistance. A dependence could, however, be shown below the discharge current of 20 m.amp and above 70 m.amp.

The curve of the amplitude of the current variations has been plotted in Fig. 4, and for a discharge current

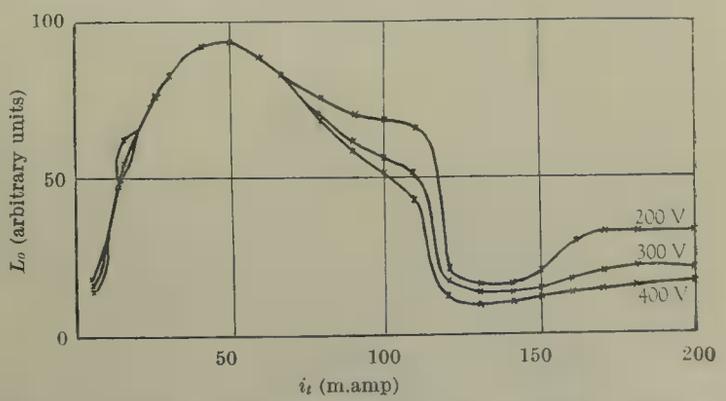

Fig. 3. The amplitude L_0 of the variation of light intensity as the function of the discharge current i_t for various supply voltages

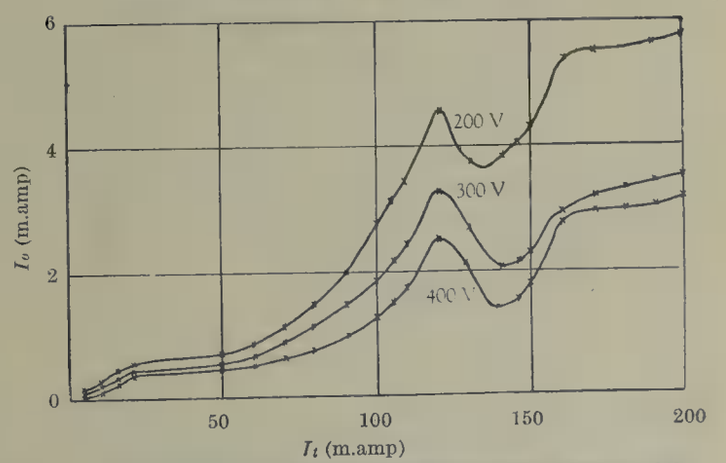

Fig. 4. The amplitude I_0 of the current variation as the function of the discharge current

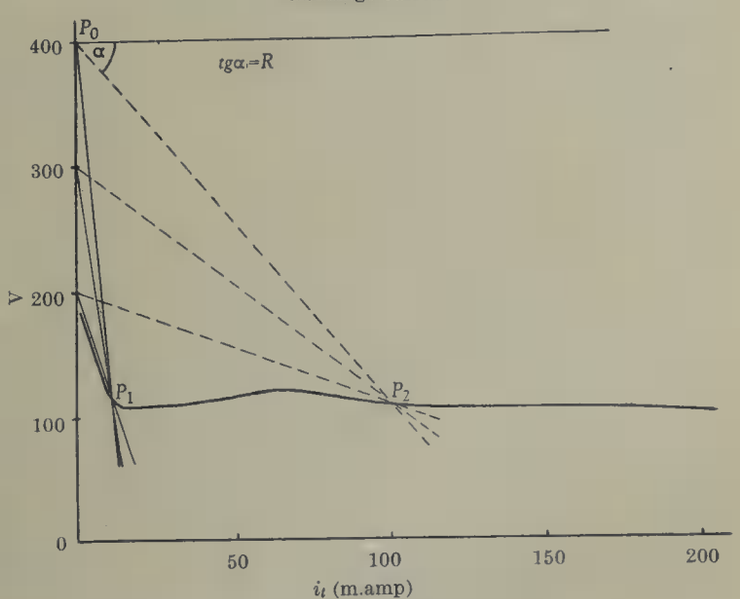

Fig. 5. Stabilization for various supply voltages

of 120 m.amp this curve has a maximum at the kink in the curve in Fig. 2.

In Fig. 5 the voltage versus current characteristic has been plotted for the gas-discharge tube under test, with the known resistance 'straights' associated with working points P_1 and P_2. At working point P_1 a steep curve section is associated with the lower current intensities. The angle closed by the resistance 'straight' and its associated characteristic section changes here much more markedly than, for example, in the neighbourhood of working point P_2. Consequently, for a given current intensity variation, a much greater voltage variation will arise, for example along the resistance 'straight' associated with the 400-V supply than along the resistance 'straight' belonging to the 200-V supply. We believe that this stronger voltage variation offers an explanation of why the frequency, as shown in Fig. 2, becomes dependent on the external parameters of the tube exactly at a current intensity of 20 m.amp corresponding to the working point P_1 in Fig. 5. This problem will receive a theoretical examination according to the method established by Kapzow[15] in the following section.

(c) *Influence of the ambient temperature on the moving striation: theoretical study of the influence of external parameters.* In Fig. 6 the discharge current I_t and the burning tube voltage V_t of the discharge tube have been plotted at a stabilized wall temperature of 25° C. It may be observed that for low current intensities the slope of the curve is extremely steep and of a negative character.

The dependence of the frequency of the moving striation on the discharge current is shown in Fig. 2 for a wall temperature stabilized at 25° C. Measurements were made in the current range 5–200 m.amp. The external limiting resistance used in the current range 5–20 m.amp had an effect on the frequency of the striation[4]. A sharp kink in the frequency curve manifesting itself at the discharge current value of 120 m.amp could be found at all three test temperatures, that is, 20°, 25° and 40° C, for the same current. The curve plotted for the current-dependence of the striation frequency followed the trend of the curve plotted in Fig. 2 for a temperature of 25° also at the other two temperatures tested.

In Fig. 7 the temperature-dependence of the frequency of the moving striation is shown for a few values of the discharge current. For currents below 120 m.amp the temperature-dependence curves have a tendency to decline; with rising wall temperatures the frequency of the striations tends to decrease. At 120 m.amp—the current at which the kink occurs in the curve plotted in Fig. 2—the temperature-dependence curve shown in Fig. 7 inverts, and for higher currents the frequency of the striation rises with rising wall temperature.

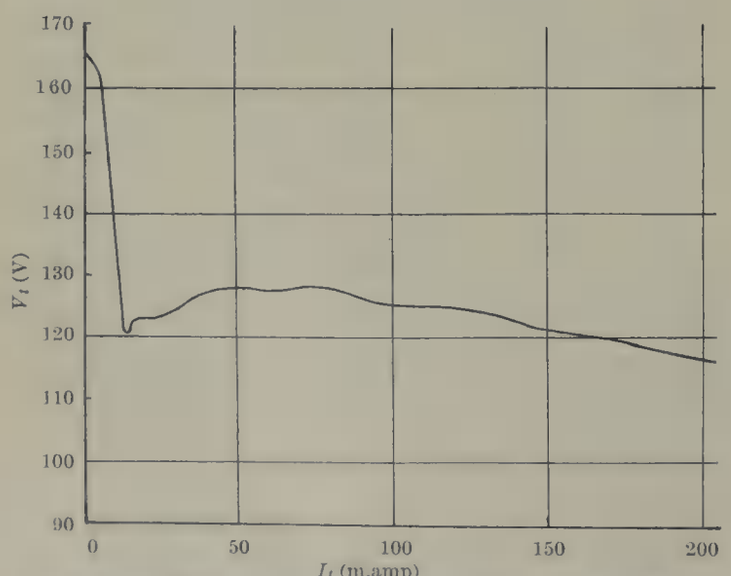

Fig. 6. Curve plotted for the discharge current, I_t, of the discharge tube versus the burning voltage, V_t

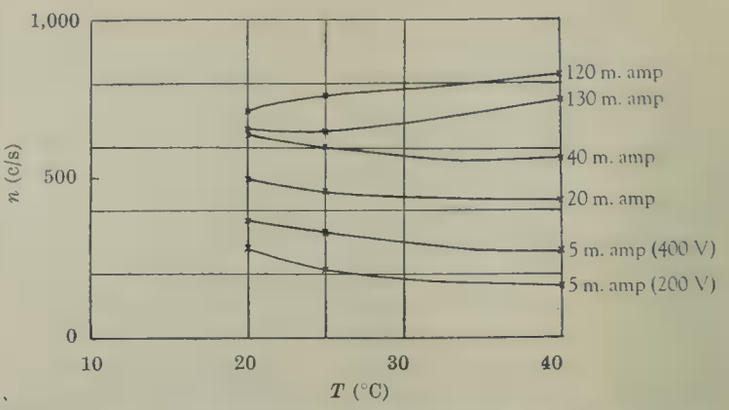

Fig. 7. Temperature-dependence of the frequency of the moving striation for specific discharge current values

As has been made clear earlier in this article, maintenance of the wall temperature at a predetermined value amounts to maintaining the concentration of the mercury atoms (which play a decisive part in the discharge) at a predetermined level. The argon atoms, owing to their higher excitation and ionization potentials, are only moderately represented in the basic processes of the discharge still in a steady-state balanced condition compared with the mercury atoms.

The values for vapour pressure and the number of mercury atoms at the various test temperatures are shown in Table 2 (ref. 7). The well-known striking growth of concentration of the mercury atoms occurs in the temperature range between 25° and 40° C.

Table 2

	°C		
	20	25	40
Vapour pressure of the mercury atoms (10^{-3} mm Hg)	1·201	1·85	6·07
Concentration of mercury atoms (atom/c.c.)	$3{\cdot}95 \times 10^{13}$	$5{\cdot}94 \times 10^{13}$	20×10^{13}

Before discussing the results obtained here, certain characteristic sections in the current versus voltage curve in Fig. 6 deserve special attention. For small currents the curve has a negative directional tangent, whereas for currents larger than 20 m.amp the curve is first positive and later negative.

The characteristics of the curve shown in Fig. 2 for the current dependence of the striation frequency can perhaps be explained with the characteristic properties described earlier and the influence of the internal parameters of the discharge. In this connexion, we outlined our opinions at the second Czechoslovak Conference on Electronics and Vacuum Physics[4], and pointed out that, whereas in the current range up to 20 m.amp the external parameters, mainly the value of the limiting resistance, affected the striation frequency in a decisive manner, for current above 20 m.amp it was mainly the internal parameters which determined the striation frequency. We also made it clear that for a theoretical description of the influence of the external factors the equivalent circuitry developed by Kapzow offered a convenient method[15]. A demonstration of this method follows later.

In all curves describing the current-dependence of the frequencies (Fig. 2) there is a break point at 120 m.amp for all three temperatures. At this point the frequency drops by 200–300 c/s, and then for currents in excess of 120 m.amp the curve progresses between values corresponding to the original frequency value and the break point, in a closely linear, monotonously rising manner up to 200 m.amp. The temperature-dependence of this frequency breakdown is represented in Fig. 7 by the curve marked with the 120-m.amp parameter.

The theoretical relationship for the influence of the ambient temperature according to Fig. 7 can only be established after further investigations. However, even without any further investigations the statement may be ventured that there may be a definite relationship between the variation of the concentration of neutral

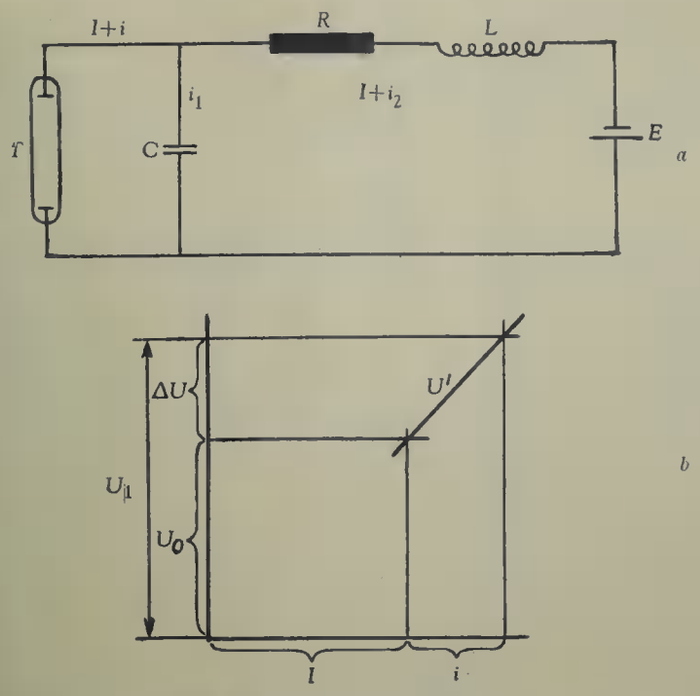

Fig. 8 (*a*) Equivalent circuitry for the electric circuit of the discharge. (*b*) Auxiliary figure for the notations used in the deduction

mercury atoms in accordance with Table 1 and the influence of the ambient temperature. A consequence of this relationship is perhaps the drop in the striation frequency with the growing mercury atom concentration below the current intensity of 120 m.amp.

From the fact that with the increase in concentration of mercury atoms the frequency variation curve becomes inverted at 120 m.amp, it can be concluded that owing to the larger energy losses associated with the larger current rate beyond 120 m.amp a change can occur in the heat dissipation conditions in the discharge. This may, among other things, influence the trend in the temperature-dependence of the moving striation frequency.

The influence of temperature should be considered as an external factor at the formation of moving striation. As has already been explained[4–6], in specific sections of the current range the external parameters resistance, capacity and induction of the electric circuit have a critical influence. A method for studying this influence has been provided by Kapzow[15]. Assuming an equivalent circuitry for the discharge circuit, he discusses the conditions for oscillation by solving the second-order differential equation for the discharge circuit.

Accordingly, for the present investigations, the electric circuit for the discharge can be described by the equivalent circuitry as shown in Fig. 8*a*, where C denotes the sum total capacity of the discharge circuit and the water-jacket surrounding the tube which affects the discharge, R is the sum total resistance of the external circuit, L is the sum total induction given by these resistances and other circuit members, and E is the voltage of the d.c. current source maintaining the discharge.

By applying Kirchhoff's law in the vibrationless, balanced state of the discharge the following relation is obtained:

$$E = I \,.\, R + U_o \tag{3}$$

where U_o denotes the tube voltage at a current I.

If with the start of an oscillatory process of any kind the current of the discharge changed to $I + i$, then corresponding to the discharge characteristic of a slope U' in accordance with Fig. 8*b*, the voltage drop of discharge would change to a value U_1. Owing to the oscillations now in full swing a current i_1 would flow through condenser C, and also the current flowing in the equivalent chain RL would vary by a value i_2.

Similar to equation (3), the following circuit relation can be written:

$$E = U_1 + R(I + i_2) + L\frac{\mathrm{d}i_2}{\mathrm{d}t} \tag{4}$$

since
$$U_1 = U_o + \Delta U \tag{5}$$

and
$$\Delta U = U' \,.\, i \tag{6}$$

further
$$U_1 - U_o = \Delta U = \frac{1}{C}\int i_1 \,.\, \mathrm{d}t \tag{7}$$

and then
$$CU'i = \int i_1 \,.\, \mathrm{d}t \tag{8}$$

$$C \,.\, U'\frac{\mathrm{d}i}{\mathrm{d}t} = i_1 \tag{9}$$

With this substituted in equation (4), since

$$i_2 = i + i_1 \tag{10}$$

the relations:

$$E = U_o + U'i + R \,.\, I + R \,.\, i + R \,.\, i_1 + L\frac{\mathrm{d}i}{\mathrm{d}t} + L\frac{\mathrm{d}i_1}{\mathrm{d}t} \tag{11}$$

and

$$E = U_o + U'i + R \,.\, I + R \,.\, i + RCU'\frac{\mathrm{d}i}{\mathrm{d}t} + L\frac{\mathrm{d}i}{\mathrm{d}t} + LCU'\frac{\mathrm{d}^2i}{\mathrm{d}t^2} \tag{12}$$

will be obtained. After turning the equations into their standard or a differential equation of the form:

$$LCU'\frac{\mathrm{d}^2i}{\mathrm{d}t^2} + (RCU' + L)\frac{\mathrm{d}i}{\mathrm{d}t} + (R + U')i + \underbrace{RI + U_o - E}_{=0} = 0 \tag{13}$$

$$\frac{\mathrm{d}^2i}{\mathrm{d}t^2} + \frac{RCU' + L}{LCU'}\frac{\mathrm{d}i}{\mathrm{d}t} + \frac{R + U'}{LCU'}i = 0 \tag{14}$$

will be obtained. After solving this equation according to:

$$i = A_1 \exp(r_1 t) + A_2 \exp(-r_2 t) \tag{15}$$

where A_1 and A_2 are constants, the following equation may be written for r:

$$r = -\frac{1}{2}\frac{RCU' + L}{LCU'} \pm \sqrt{\frac{1}{4}\left(\frac{RCU' + L}{LCU'}\right)^2 - \frac{R + U'}{LCU'}} \tag{16}$$

or in a reduced form:

$$r = -\frac{1}{2}\left(\frac{R}{L} + \frac{1}{CU'}\right) \pm \sqrt{\frac{1}{4}\left[\frac{R}{L} + \frac{1}{CU'}\right]^2 - \frac{1}{LC}\left[\frac{R}{U'} + 1\right]} \tag{17}$$

(*a*) If the quantity under the root sign is positive, then the current variation is of an aperiodic nature, and r_1 and r_2 are real. In this event the condition for stability may be written:

$$r_1 < 0 \tag{18}$$

$$r_2 < 0 \tag{19}$$

This condition can be satisfied, first, if

$$\frac{R}{L} + \frac{1}{CU'} > 0 \tag{20}$$

and, secondly, if the absolute value of the first term under the root sign is smaller than the square of the first term. Otherwise a positive r would also be possible, that is, to satisfy conditions (18) and (19) it is required that:

$$\frac{1}{4}\left(\frac{R}{L} + \frac{1}{CU'}\right)^2 - \frac{1}{LC}\left(\frac{R}{U'} + 1\right) < \left[\frac{1}{2}\left(\frac{R}{L} + \frac{1}{CU'}\right)\right]^2 \tag{21}$$

that is, the inequality:

$$\frac{1}{LC}\left(\frac{R}{U'} + 1\right) > 0 \tag{22}$$

exists, since both L and C are positive, for real r_1 and r_2 the condition for stability is given in the form:

$$\frac{R}{U'} + 1 > 0 \qquad (23)$$

(*b*) If the quantity under the root sign in equation (17) is negative, then a solution will be found in the form:

$$i = A_1 \exp(-\delta t + j\omega t) + A_2 \exp(-\delta t - j\omega t) \qquad (24)$$

where A_1 and A_2 are constants, and

$$\delta = \frac{1}{2}\frac{R}{L} + \frac{1}{CU'} \qquad (25)$$

$$j\omega = \sqrt{\frac{1}{4}\left(\frac{R}{L} + \frac{1}{CU'}\right)^2 - \frac{1}{LC}\left(1 + \frac{R}{U'}\right)} \qquad (26)$$

The condition for stability is that $\delta > 0$, that is, the expression in (25) must also be greater than zero. Thus the inequality:

$$\frac{1}{2}\left(\frac{R}{L} + \frac{1}{CU'}\right) > 0 \qquad (27)$$

should exist. If conditions (22) and (26) exist at the same time, then the external parameters will cause no instability in the discharge.

By using relation (26) the oscillation frequency in the neighbourhood of the current value corresponding to the working point may be given which might then become the generator of the moving striation.

With the values of the external circuit elements in (26) and the slope of the curve associated with the current value in question inserted, a quantitative relation will be obtained showing that the frequencies of the oscillations are in fact within the range between 100 and 1,000 c/s.

In the course of the experiments reported here the ohmic resistance, capacity, and inductance of the external electric circuit were determined for the purpose of these calculations. The latter inductance is due to the inductivity of the ohmic resistance.

As an external value the capacity of the water-jacket surrounding the discharge tube had to be taken into account. With this capacity taken up in the calculation the values of all other capacitative elements of the circuit become negligible. In Table 3 the circuit parameters associated with a 50-m.amp discharge current and the value of the slope U' taken from Fig. 6 have been compiled.

Table 3

R	L	C circuit	C jacket	U'
3,440 ohms	12×10^{-3} henries	14×10^{-12} farads	$2{\cdot}1 \times 10^{-8}$ farads	150 ohms

The value of the frequencies is markedly influenced by the values of U' and R, whereas L varies only slightly, and C may in this case be considered a constant (water-jacket). Depending on the slope and the character of the curve a variety of frequency values will be obtained from equation (26) for the various currents. As for order of magnitude, these frequency values agree with the experimentally established frequencies.

It is thus obvious that external parameters (especially temperature) together with the members of the external electric circuit have a critical influence on the striation processes. In the course of pertinent investigations more extensive and far-reaching information may be obtained concerning the fundamental physical phenomena occurring in the plasma.

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KINETIC PARAMETERS FOR OXYGEN IN REACTIONS INVOLVING PURIFIED OXIDASES AND TISSUE SLICES

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HAYAISHI[1] has recently summarized the apparent Michaelis constants for oxygen of several oxidase enzymes. In only one of the papers cited (that by Longmuir[2]) was the time-course of the reactions considered in any detail. In order to assess the suitability of Longmuir's polarographic apparatus to our own work, we have examined the applicability of both integrated and differential rate methods for determining the kinetic parameters for oxygen from the time-course of two oxidase reactions. These procedures are basically inferior to those in which only initial rates are considered due, for example, to effects resulting from the accumulation of products. However, we have found that consistent results are obtainable by these methods, provided excess catalase is added to decompose rapidly hydrogen peroxide formed during the reactions considered. The good reproducibility of the values obtained for the parameters invites a more frequent use of these methods especially when the supply of enzyme is limited and maximum information must be gleaned from each individual experiment. Application of these methods to the results obtained on the oxygen uptake by intact cells (cf. Longmuir[3], Chance[4], Bänder and Kiese[5]) leads us to propose an alternative interpretation of published results on the kinetics of tissue respiration.

The apparatus used for these investigations was essentially as described by Longmuir[2], the cell volume being approximately 18 ml. The experimental technique adopted was as follows: a buffered solution of the primary substrate (in large excess) was equilibrated with air (or oxygen at up to 3 atmospheres pressure) at 37° C, this

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being followed by the addition of excess catalase (final concentration approx. 5 μg/ml.). The latter had no effect on the diffusion current. The mixture was then introduced into the air-tight polarographic cells (maintained at the same temperature) and the reactions were initiated by the injection of 0·2 ml. of the appropriate enzyme solution. Glucose oxidase was obtained from Boehringer and Soehne, Mannheim, Germany. This enzyme is specific for β-D-glucose, but, since the preparation contained a highly active epimerase, the β-D-glucose concentration was effectively that of the total D-glucose present. Pig kidney D-amino-acid oxidase was purchased from Seravac Laboratories, Ltd., Colnbrook, England, the substrate used being D-alanine.

Analysis of the results obtained with both glucose oxidase and D-amino-acid oxidase shows that, under the conditions described, the reactions conform to simple Michaelis–Menten kinetics except at extreme values of oxygen concentration. Thus, if the data are plotted in the form of the integrated rate equation:

$$\frac{S_0 - S}{t} = V_m + \frac{K_m}{t} \cdot \ln\left(\frac{S}{S_0}\right)$$

(where S_0 and S are the oxygen tensions at time zero and time t respectively, V_m is the theoretical limiting rate and K_m the apparent Michaelis constant), the graphs obtained are linear over a considerable range of oxygen pressures (for example, 30–700 mm mercury). From the intercepts and slope it is thus possible to calculate both V_m and K_m. Alternatively, but perhaps less accurately, it proved possible to determine the rate of the reaction at any given partial pressure of oxygen from the slope of the time-course trace and hence obtain Lineweaver–Burk plots. The kinetic parameters can then be obtained in the usual way. In addition it is possible to determine the apparent reaction order for oxygen from the slope, n, of the log $\left(\frac{V_m - V}{V}\right)$ versus log S plot (where V is the rate at the oxygen tension S). This value should be unity for simple Michaelis–Menten kinetics. By these methods the following range of values were obtained from the data of five experiments with each enzyme.

Glucose oxidase: V_m = 240–270 μl. O_2/min (mean 250); K_m (oxygen) = 120–190 mm mercury (mean 150): n = 0·97–1·05 (mean 1·00) (0·05 M phosphate buffer, *p*H 6, 0·01 M potassium chloride, 0·05 M glucose; initial oxygen tension varied from air saturated to 1 atmosphere).

D-Amino-acid oxidase: V_m = 85–95 μl. O_2/min (mean 89); K_m (oxygen) = 120–130 mm mercury (mean 125); n = 0·94–1·03 (mean 0·98) (0·05 M phosphate buffer, *p*H 8, 0·01 M potassium chloride, 5×10^{-2} M D-alanine; initial oxygen tension varied from air saturated to 3 atmospheres).

The K_m values in both cases (equivalent to about 170 μM for D-amino-acid oxidase and 210 μM for glucose oxidase) are in good agreement with the values obtained by other methods (140–300 μM and 200 μM respectively[6,7]).

Non-conformation at both high and low oxygen tensions is to be expected as a result of the inapplicability of the simple theoretical equations at extreme values. In addition the degree of accuracy of the analytical methods is also lower in these regions. The deviations from simple Michaelis–Menten kinetics observed at very high oxygen tensions appear, however, to be real and could be due to inhibitions by oxygen or to intermediate intra-enzymatic reactions (that is, those not directly involving oxygen), or to the primary substrate being rate-limiting.

The calculations described were also applied to results obtained with actively respiring rat liver slices (using succinate as substrate) in place of the purified oxidase systems. Again the kinetics of the uptake of oxygen conformed to simple Michaelis–Menten kinetics over a wide range of oxygen tensions. Measurements of this kind have been conducted by several workers[2–4]: each of these have referred to the apparent reaction order for oxygen as being anomalous, with a non-integral value in the region of 1·3–1·4. It should be pointed out, however, that these workers all used the *observed* maximal rate for the value of V_m. If, on the other hand, one uses the *theoretical* value for V_m (for example, the value obtained from extrapolation of the Lineweaver–Burk plots) as required by the relevant equations, a reaction order of unity is obtained (Fig. 1). It should be noted that the linearity of the Lineweaver–Burk plot implies a reaction order of unity irrespective of the nature or value of the maximum velocity. In the demonstration of the reaction order by means of the log–log plot, however, the interpretation will be dependent on the value used for the maximum velocity. Similarly, if the observed and theoretical limiting rates are significantly different, then large errors may be incurred in the estimation of the K_m value from the oxygen concentration at which the respiration is half-maximal. The anomaly can thus be resolved on the basis that the observed and theoretical maximum rates are not necessarily equivalent. On this interpretation, it would appear that, under the conditions used, the reactions directly involving oxygen are not initially rate-limiting but become so when the tension falls below the so-called critical value. Chance[4] has described how the tissue respiratory data can be explained on the basis of sequential reactions involving four cytochrome components and this rationale is unaffected by our interpretation of the data.

In addition, we wish to mention that the apparent Michaelis constants for oxygen which we have obtained for tissue respiration (200 mm mercury, about 300 μM) differ by as much as one or even two orders of magnitude from those hitherto reported for the reaction of oxygen with rat liver cytochrome oxidase[1]. It thus seems necessary to contemplate the existence of other rate-determining factors.

Some anomalies have already been considered by Longmuir and Bourke[8,9]. Initially they felt the discrepancy might be explained by a gradient of partial pressure of oxygen through tissue down which oxygen diffused from the outside to the respiratory site within the tissue. However, this required a value for the diffusion coefficient for oxygen in tissue some hundred-fold less than through water. In addition, their original hypothesis did not explain the paradoxical result that the thickness of the slice appeared to have little effect on the parameter of critical oxygen tension of the tissue: the existence of a diffusion gradient would require that the critical oxygen

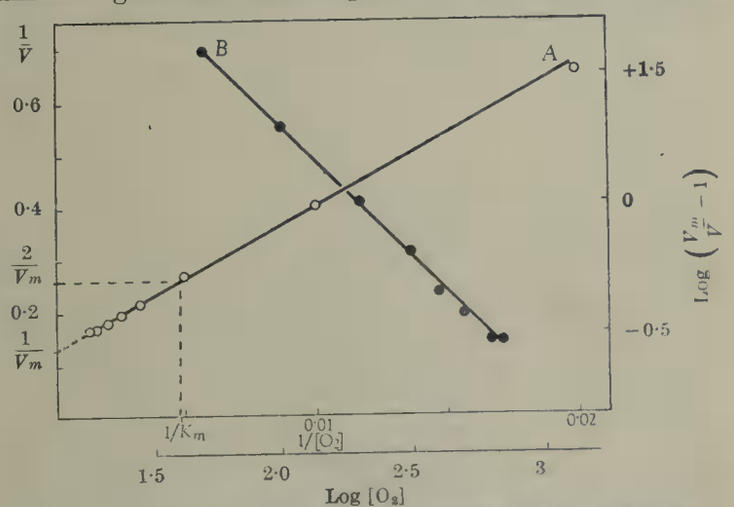

Fig. 1. Respiration of rat liver: kinetic parameters for oxygen. A freshly prepared slice (0·4 mm thick) of rat liver was suspended in a medium (0·05 M phosphate buffer *p*H 7·4, 0·05 M KCl 37° C) contained in a reaction cell of the Longmuir type. Respiration was initiated by injection of sodium succinate to a final concentration of 0·12 M. Curve *A* shows the conformation to simple Michaelis–Menten kinetics by means of the Lineweaver–Burk plot: this graph also indicates the method of determining the theoretical maximum rate (V_m, 73 μl./min) and the Michaelis constant (K_m, 220 mm mercury). The slope of the double logarithmic plot (curve *B*) gives the reaction order for oxygen (1·0)

tension should increase as the square of the slice thickness. Further examination of their experimental results led Longmuir and Bourke to the conclusion that diffusion could not be limiting, indeed the experimentally determined value of the diffusion coefficient for respiratory tissue appeared to be 10 times that for water. In addition, they considered that respiration of a slice might be limited by an oxidase with a low affinity for oxygen[10].

The differences noted could arise from the existence of an active transport mechanism for oxygen within the cell. On the other hand, the results may merely reflect a marked dependence of the parameters on the conditions used, an alteration in the kinetic behaviour of the cytochrome oxidase as a result of cell disruption or perhaps the existence of different kinetic forms (isozymes) of the latter enzyme within a particular species.

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URINARY METABOLITES IN THE ω-OXIDATION OF 2,4-DIMETHYLHEPTANOIC ACID

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WHILE examining the metabolism of 2,4-dimethylheptanoic acid-2-methyl-^{14}C (DMHA) in the intact rat, it was found that approximately 20 per cent of the radioactive label was recoverable in the urine after 48 h, while more than 60 per cent was recoverable in respiratory carbon dioxide. (Manuscript in preparation. Normally, less than 1 per cent of the radioactivity from a carbon-14 labelled fatty acid is excreted in the urine.) An investigation of the nature of this radioactive material indicates that it may be the ω-oxidation product of the parent compound.

ω-Oxidation of fatty acids occurs when oxidation from the carboxyl end of the molecule is blocked, or when the acid is of medium chain-length[1,2]. Tryding and Westoo examined a series of ^{14}C-carboxyl labelled, methyl-substituted stearic acids[3] which indicate that α-substitution has little overall effect on metabolism, while β- or α,α-disubstitution almost completely block β-oxidation. The demonstration of ω-oxidation *in vitro* in a cell-free system has also been reported[4]. On the other hand, there is no previous report concerning the metabolism of methyl-substituted fatty acids such as DMHA in which the substitution occurs on the α and γ carbon atoms.

Rats weighing 200 g were given a diet containing 10 per cent corn oil, and 0·2 per cent of DMHA-2-methyl-^{14}C, specific activity 5 × 10^4 d.p.m./mg. The synthesis of the non-radioactive compound has been previously reported[5]. The radioactive label was incorporated through a minor variation of the published procedure which involved the condensation of diethyl 2-methylpentylmalonate[5] with methyl-^{14}C-iodide. The rats were housed in glass metabolism cages and urine was collected over a period of 48 h. The urine was acidified to *p*H 1, and continuously extracted with ether for 18 h with and without prior saponification with potassium hydroxide. Under these conditions, 93 per cent of the urine radioactivity (by liquid scintillation counting) could be recovered from the saponified urine, while only 57 per cent could be recovered from the unsaponified urine, indicating the presence of conjugated material.

Acids in the ether extract from the saponified urine were esterified with diazomethane[6] and gas-chromatographed on a 2-meter column of 10 per cent diethyleneglycol succinate on acid-washed diatomaceous earth at a helium flow rate of 50 ml./min. The chromatographic effluent corresponding to each major peak in the chromatogram was collected in glass capillaries immersed directly in liquid scintillation counting vials containing the scintillation liquid. Only three major peaks were found which did not correspond to a chromatogram of the urine of rats which had been fed the same diet without DMHA. These peaks were designated as shown in Table 1. None of these peaks had the same retention time as the methyl esters of a series of known compounds. (Methyl esters of: C-2, C-3, C-4, C-6, C-7, C-10 straight-chain dicarboxylic acids; methylmalonic, methylsuccinic, 2-methylglutaric, 3-hydroxybutyric, lactic, malic and citric acids. Ketoacids and fumaric acid are excluded, since they are not satisfactorily esterified and give non-volatile products with diazomethane.) The only compound which occurred in normal urine, and had a significant fraction of radioactivity, was dimethyl succinate (Table 1). No unmetabolized DMHA could be detected, although the method is suitable for identification of 5 μg of this compound.

Table 1. Gas Chromatography of Esterified Acidic Components of Urine of Rats fed 2,4-Dimethylheptanoic Acid

Peak	Retention time (min)*	Percentage of total urine radioactivity
A	76	21·8
B	70	35·5
C	56	7·4
Dimethyl succinate	16	2·3
Methyl 2,4-dimethylheptanoate	8·5	0

* A 2-meter column of 10 per cent diethyleneglycol succinate on acid-washed diatomaceous earth. Column temperature 100° C. Helium flow rate 50 ml./min.

The materials corresponding to peak *A*, *B*, and *C* were trapped from the chromatograph in 1-ml. conical centrifuge tubes cooled with liquid nitrogen. Their infra-red spectra were determined in a Beckman *IR*-5 spectrophotometer using 0·05-mm cavity cells and a 5 × KBr beam condenser. The spectra of peaks *A* and *B* were essentially identical both as pure liquids and in CCl_4 solution. This supports an assumption that they are diastereoisomers, as had previously been demonstrated for the parent compound, DMHA[5]. The spectra of *A* and *B* indicated that these compounds were esters. The transmission ratio of the carbonyl band at 5·75μ to that of the C—H band at 3·75μ was similar to that found in a series of dicarboxylic rather than monocarboxylic esters, suggesting that *A* and *B*

might be dicarboxylic esters. The presence of a band at 7·25μ also indicated the possibility of methyl groups on the chain[7], particularly because the retention time did not correspond to that of a straight-chain compound. Although the specific activities of *A* and *B* could not be accurately determined owing to the very small amount of material available, on a volumetric basis, they appear to be in the range of 90 per cent of that of the parent compound, DMHA. It was tentatively assumed, therefore, that *A* and *B* were derived from DMHA via ω-oxidation and had the structure of 2,4-dimethylpimelic acid (1).

$$HO_2C—CH_2—CH_2—\underset{\displaystyle CH_3}{\underset{|}{CH}}—CH_2—\underset{\displaystyle CH_3}{\underset{|}{CH}}—CO_2H \qquad (1)$$

The mass spectrum of *B* was obtained with a CEC 21–130 mass spectrometer, and indicated fragment peaks typical of dicarboxylic esters[8,9]. The peak at highest mass to charge ratio obtained was at m/e 185, and no discernible peak could be seen at m/e 216 where the parent mass ion (*M*) might be expected. It would not be unusual to find no parent mass ion for this type of compound, and the peak at m/e 185 probably corresponds to loss of a methoxyl ion (*M*-31). The base peak in the spectrum was at m/e 55 (C_4H_7), and corresponds to the branched paraffinic ion expected from the fragmentation pattern of (1). Other important peaks were found at m/e 88 (re-arrangement typical of 2-methyl substituted methyl esters (ref. 9)), m/e 59 (CO_2CH_3), and m/e 83 (C_6H_{11}, fragmentation at 1,2 and 5,6 with loss of H). No major peaks were detected that would not logically arise from (1). It is therefore tentatively concluded that (1) is the correct structure for *A* and *B*.

The infra-red spectrum of peak *C* was similar to that of DMHA, both in the position and intensity of the bands over the region of 3·3–9·5μ. The major differences in the spectra of *C* and DMHA are the presence in the spectrum of *C* of a strong band at 2·9μ and a medium intensity band at 10·6μ. The 2·9-μ band is due to a hydroxy group and varies in position with dilution as expected[10], shifting to lower wave-lengths in more dilute solutions. The 10·6-μ band may indicate the type of substitution of the carbon bonded to the hydroxyl, but is of limited value[10]. Since only a trace of this compound was available, it was not possible to obtain any further information regarding its structure. On the basis of biological considerations, *C* might be the β-hydroxy derivative of DMHA, a compound which would be a normal intermediate of β-oxidation. Another possibility is that *C* is the ω-hydroxy derivative of DMHA, since it has recently been shown that the ω-hydroxy acid is an intermediate in ω-oxidation[4].

It might have been expected *a priori* that fatty acids with alternate α-methyl substitution would be metabolized *in vivo*, via β-oxidation, with the same ease that has been demonstrated for fatty acids with a single methyl group in the 2 or 4 position[3]. Although 60 per cent of the ingested DMHA was oxidized to carbon dioxide via β-oxidation, the presence of 20 per cent of the radioactivity in urine might indicate that the alternate methyl groups along the chain present increased steric hindrance at one of the active sites in the β-oxidation enzyme system. Alternatively, it is possible that the extent of ω-oxidation is primarily a function of the chain-length of the compound —a hypothesis which remains to be tested.

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'PINK SPOT' IN THE URINE OF SCHIZOPHRENICS

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SINCE Friedhoff and van Winkle[1] reported that they had isolated the compound 3,4-dimethoxyphenylethylamine (DMPE)—the 'pink spot'—from the urine of 15 out of 19 schizophrenics whereas they found it was absent in 14 mentally normal people, other workers have carried out similar investigations. Of these, Sen and McGeer[2], Kuehl *et al.*[3] and Horwitt[4] were confirmatory, Perry *et al.*[5] and Faurbye and Pind[6] were not, and Takesada *et al.*[7] found that the compound was also present in nearly half their controls. In all these series the number of individuals tested was small, and moreover it appears that in none of them were the tests done in ignorance of the diagnosis. In the past many metabolic abnormalities have been described in schizophrenia, but none has since been shown to have any fundamental bearing on the disease. It therefore seemed important to us to investigate the pink spot further, for if it could be shown to be causally related to schizophrenia this would provide important evidence favouring the abnormal methylation hypothesis as put forward by Osmond and Smythies[8] and Harley-Mason[9].

We have tested for the pink spot in the urine of 808 individuals to try to find out how often it is found : (*a*) in the different forms of schizophrenia; (*b*) in different types of mental disease; (*c*) in close relatives of schizophrenics; (*d*) in mentally normal people. To try to answer these problems, four experiments were planned:

Exps. I and II : Here two independent and 'blind' surveys were carried out in which separate workers ascertained the incidence of the pink spot using different

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methods for its detection and also different criteria for the assessment of schizophrenia.

Exp. III: A small series of family studies in which the close relatives of pink spot positive schizophrenic propositi were investigated.

Exp. IV: A survey of mentally normal individuals some of whom were apparently healthy in every way and some of whom were in-patients in the wards of two general hospitals.

Exp. I. The urines of 101 mental hospital in-patients, all 'possible schizophrenics', were tested with the primary object of finding the incidence of pink spot in the disease. The patients, who were drawn from three mental hospitals, consisted of acute and chronic cases and some had been off drug therapy for up to two months. All had at one time been given a provisional diagnosis of schizophrenia, and this and the drug therapy were known to the investigator (R. E. B.) who was testing for the pink spot. After he had assessed the presence or absence of this, each patient was interviewed and re-assessed by a psychiatrist (S. A. L.) who was ignorant of the pink spot finding. It was she who made the final assessment, and in about 17 per cent of the patients she thought the original diagnosis of schizophrenia was substantially in doubt; when this was so they were classed as non-schizophrenic. Where the diagnosis of schizophrenia was definitely upheld the patients were grouped into those who at any time had exhibited one or more of Schneider's first-rank symptoms[10] and those who had not (these are referred to as Schneider positive and Schneider negative respectively). Afterwards the patients were also sub-divided into classical schizophrenics and those who showed only paranoid features without formal thought disorder or flattening of affect. The former for convenience are referred to as 'non-paranoid' and the latter as 'paranoid'. In five patients the information necessary for diagnosis was unobtainable either because the patients were unco-operative or because of language difficulties, and these five are classed as 'impossible to assess'.

The urine sample was, whenever possible, collected over 20 h, though in some cases shorter periods of collection proved more practicable. The extraction and the paper chromatography were carried out as described by Friedhoff and van Winkle[1] and this is referred to here as Method 1. Where there was marked interference by drugs the presence or absence of the pink spot sometimes had to be classed as 'impossible to assess'.

Because a much more detailed investigation would be necessary to ascertain that 3,4-DMPE was in fact being demonstrated we have, throughout this article, preferred to use the term 'pink spot' rather than 'presence of DMPE' in reporting the results.

These are given in Table 1 and the statistical analysis in Table 2. This consists of various comparisons using the χ^2 test or Fisher's two-tailed test for exact probability, whichever seemed the more appropriate. The control group were 149 mentally normal individuals (tested by Method 1) none of whom had the pink spot. Given also (Table 3) are the percentages of individuals with the pink spot within sub-classes.

Table 1. Experiment I

(*a*) Pink spot in relation to Schneider's first-rank symptoms

	Pink spot rating			
Schneider rating	Positive	Negative	Impossible to assess	Totals
Positive	35	11	6	52
Negative	7	16	4	27
Impossible to assess	4	0	1	5
Non-schizophrenic	0	16	1	17
Totals	46	43	12	101

(*b*) Pink spot in relation to non-paranoid and paranoid classification

Psychiatric assessment		Pink spot rating: Positive	Negative	Totals
Schneider positive	Non-paranoid	35	7	42
	Paranoid	0	4	4
Schneider negative	Non-paranoid	5	5	10
	Paranoid	2	11	13
	Totals	42	27	69

Table 2. Experiment I. Comparison of Incidence of Pink Spot between Classes

Comparison	χ^2	d.f.	Probability
Schneider positive with Schneider negative	11·57	1	<0.001
Schneider positive with non-schizophrenic	24·95	1	<0.001
Schneider positive with control			$=2.5\times10^{-10}$
Schneider negative with non-schizophrenic			$=0.029$
Schneider negative with control			$=3.1\times10^{-7}$
Non-paranoid with paranoid	20·18	1	<0.001
Non-paranoid with non-schizophrenic	26·80	1	<0.001
Non-paranoid with control			$=8.06\times10^{-32}$
Paranoid with non-schizophrenic			N.S.
Paranoid with control			$=0.0099$
Schneider positive with Schneider negative within non-paranoid			$=0.0388$
Schneider positive with Schneider negative within paranoid			N.S.
Paranoid with non-paranoid within Schneider positive			$=0.0020$
Paranoid with non-paranoid within Schneider negative			N.S.

Table 3. Experiment I. Percentage of Individuals with Pink Spot within Sub-classes

Sub-class	Percentage with pink spot
Schneider positive, non-paranoid	83.3 ± 5.75
Schneider positive, paranoid	0·0
Schneider negative, non-paranoid	50 ± 15.8
Schneider negative, paranoid	15.4 ± 10.0

Exp. II. In the second experiment a different psychiatrist (P. H.) and biochemist (A. P. R.) worked together. The urines of 296 psychiatric in-patients drawn from two hospitals were investigated for the pink spot. The patients had often been in hospital for many years and included a larger proportion of chronic cases than in the first experiment. The samples of urine were analysed in ignorance both of the diagnosis and of the nature and quantity of drugs being received, so that a different part of the work was 'blind' from that in Exp. I. The psychiatrist selected the patients so as to include a variety of mental diseases, and he accepted the diagnosis given in the case-sheets and did not interview the patients personally as did the psychiatrist in Exp. I. He divided the cases into four groups: (*a*) 'non-paranoid' schizophrenics; (*b*) 'paranoid' schizophrenics; (*c*) schizophreniform syndromes (where although some features of schizophrenia were present it was uncertain whether or not the primary diagnosis was schizophrenia); (*d*) non-schizophrenics, which included such conditions as manic depressive psychosis, organic dementia and mental defect.

Since a different psychiatrist was scoring the cases and the diagnosis was assessed from the case sheets, the classes are probably not strictly comparable with those of Exp. I.

The chromatographic procedures were the same as those described by Friedhoff and van Winkle[1] (our 'Method 1'), but a different extraction procedure was used and the technique is referred to by us as 'Method 2'. A volume of 300–500 ml. of a 16-h overnight sample of urine was adjusted to *p*H 9·0 with 2 N sodium hydroxide. This was extracted three times with 100-ml. portions of 1,2-dichloroethane and the extracts dried over anhydrous sodium sulphate. The first extract was examined by ultra-violet absorption at 279 mμ. A peak at this value is a useful indicator of DMPE in the absence of interfering substances such as drug metabolites. The ultra-violet absorption spectrum helps particularly to distinguish DMPE from any closely related compounds which could not satisfactorily be resolved from it by the chromatographic procedures used. The three extracts were then combined and evaporated to dryness on a rotary evaporator. The residue was

Table 4. Experiment II. Pink Spot Assessment in 296 Psychiatric Patients

	Pink spot rating			
Psychiatric assessment	Present	Absent	Impossible to assess	Totals
Non-paranoid schizophrenics	20	30	19	69
Paranoid schizophrenics	2	54	6	62
'Schizophreniform syndromes'	5	58	25	88
Non-schizophrenics	1	68	8	77
Totals	28	210	58	296

Table 5. Experiment II. Comparison of Incidence of Pink Spot between Classes

Comparison	χ^2	d.f.	Probability
Non-paranoid with paranoid	19·16	1	< 0·001
Non-paranoid with non-schizophrenic	27·05	1	< 0·001
Non-paranoid with control			$= 2{\cdot}81 \times 10^{-18}$
Paranoid with non-schizophrenic			N.S.
Paranoid with control			N.S.

redissolved in dichloroethane for application to the chromatography paper. Authentic 3,4-dimethoxyphenylethylamine (K and K Laboratories) and mixed spots of urine extracts and authentic DMPE were applied alongside the urine extracts on the chromatography papers and the separation carried out.

The results of Exp. II are given in Table 4 and the statistical analysis in Table 5. The controls are 310 mentally normal individuals (tested by Method 2) one of whom had the pink spot. The results for the schizophreniform syndrome class have not been analysed because the group was probably very heterogeneous.

Exp. III. The pink spot was investigated in 20 close relatives of three schizophrenic propositi, all three of whom were found (by one or both of the two methods) to excrete the pink spot. None of the relatives was schizophrenic though some were reported as having other mental abnormalities.

The pink spot was not found in the urine of any of the close relatives, and these therefore are not significantly different from the control group of mentally normal individuals.

Exp. IV: Investigation of mentally normal individuals. These consisted of two groups. The first was made up of 265 healthy individuals, mostly undergraduates or university staff. The second was composed of 126 mentally normal in-patients, comprising 54 pre-operative and 20 post-operative cases, 10 patients with liver disease, 20 with chronic neurological states and 22 with general medical conditions. The pink spot was assessed by one or both of the two methods previously described and the series has acted as the control for the various comparisons.

All the controls were negative for the pink spot with the exception of one healthy individual, a woman aged 54. She suffered from migraine but was mentally normal and so was her family.

Comparison between Method 1 (Friedhoff and van Winkle) and Method 2 (ultra-violet + chromatography) in the investigation of the pink spot. In 133 people both methods for assessing the pink spot were used (Table 6).

Table 6. Comparison of Method 1 and Method 2 in Investigation of the Pink Spot

	Ultra-violet + chrom. (Method II)		
Friedhoff and van Winkle (Method 1)	Pink spot positive	Pink spot negative	Totals
Pink spot positive	12	7	19
Pink spot negative	2	112	114
Totals	14	119	133

Though there is a significant correlation, $P = 1{\cdot}06 \times 10^{-10}$, between the results given by the two methods, there are discrepancies.

There are several reasons which might account for this non-concordance: (1) Method 2 may be less sensitive in detecting the pink spot (as opposed to DMPE); (2) slightly different substances may be being estimated; (3) one method may be more likely to produce undetectable false readings because of the presence of drugs; (4) there may be observer discrepancies. These possibilities are being investigated and will be the subject of a further article.

The association previously found between the pink spot and schizophrenia has been confirmed using large numbers. The results of Exp. I give compelling evidence that its presence is particularly associated with Schneider-positive individuals. It is much rarer in those who are Schneider negative and rarer still (if present at all) in non-schizophrenics.

In Exp. I the same patients have also been classified as to whether or not they only have paranoid features. The presence of the pink spot is particularly associated with the non-paranoid group but is significantly higher among the paranoid group than among the mentally normal controls.

When the individuals are classified as being Schneider positive and non-paranoid this sub-class has a significantly higher incidence of the pink spot than does any other group ($83{\cdot}3 \pm 5{\cdot}75$ per cent). Thus to predict the presence of the pink spot the double classification is the most efficient.

Five individuals were so seriously disturbed that they could not be interviewed or assessed on the Schneider rating. The fact that four of them showed the pink spot suggests that these were true Schneider-positive non-paranoid cases as judged by our other results.

The second experiment using a different method for detecting the pink spot again shows the very strong association between the presence of the pink spot and 'non-paranoid' schizophrenia. The lower frequency of the pink spot may be due to any of the four reasons mentioned above and/or to the cases being less florid than in Exp. I. However, not enough have been scored by the Schneider rating to show whether the association is particularly with the Schneider-positive 'non-paranoid' group although the data show a strong tendency in this direction.

The evidence from the two experiments suggests that the pink spot is highly associated with schizophrenia and is not often present in other forms of mental disorder or in close relatives or in controls. Moreover, from our survey it seems highly improbable that diet and institution life play any part in producing the pink spot. An important problem is whether the abnormal metabolite precedes or is a result of the disease. The family data point to the latter conclusion, but it is possible that the pink spot only appears shortly before the disorder manifests itself, and that more refined techniques might demonstrate it in sibs. It would be interesting to follow pink-spot-positive patients with florid schizophrenia during remissions since these data might indicate whether the acuteness of the disease was associated with the quantity of pink spot material. If it did, this might explain its absence in sibs. The possibility has to be considered that drugs might be the cause of the pink spot. This is refuted by our data. Thus the relationship between schizophrenia and pink spot is at least as marked in those who had been off drugs for two weeks or more as in those who had not. Moreover, the non-schizophrenics on drugs showed no elevation of the frequency of the pink spot over those not on drugs. However, a complicating factor in using the pink spot for diagnosis is that drugs interfere both with the chromatography and with the ultra-violet absorption and it is difficult to obtain patients (particularly acute cases) who are not on drug therapy.

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INTERFERON APPEARANCE STIMULATED BY ENDOTOXIN, BACTERIA, OR VIRUSES IN MICE PRE-TREATED WITH *Escherichia coli* ENDOTOXIN OR INFECTED WITH *Mycobacterium tuberculosis*

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WHEN mice are injected intravenously with bacteria or bacterial endotoxin an inhibitor indistinguishable from virus-induced interferon appears in the circulation[1]. *Salmonella typhimurium*, *Serratia marcescens*, and endotoxin from *Escherichia coli* produce peak-levels of circulating interferon at about 2 h after injection. Maximum interferon-levels following injection of *Brucella abortus* or Newcastle disease virus (NDV) are reached at about 12 h. To elucidate the mechanisms of the different time patterns of appearance of interferon, experimental procedures known to alter the reactivity of animals to the lethal or pyrogenic effects of endotoxin were investigated. Repeated exposure to endotoxin reduces the responsiveness of animals to subsequent injection of endotoxin, while prior infection with *Mycobacterium tuberculosis* is known to increase the reactivity of the host to endotoxin. These phenomena have been termed 'tolerance' and 'hyper-reactivity' in the literature dealing with endotoxin[2]. In connexion with the investigations reported here, which primarily deal with interferon production rather than lethality or pyrogenicity, it seems more appropriate to use the term 'hyporeactivity' in place of 'tolerance'. This article deals with the modification of interferon appearance in the circulation which results from prior treatment of mice with endotoxin or infection with tubercle bacilli.

Methods for the inoculation and bleeding of mice, for the assay of interferon in *L*-cells using the plaque-reduction method with vesicular stomatitis virus (VSV), and for the preparation and standardization of inocula containing *B. abortus* or NDV have been described in detail previously[1,3]. The endotoxins used were prepared from different strains of *E. coli* (0111 : B4, 0113, or K235) and had similar potencies when measured by LD_{50} determinations or interferon appearance in the plasma. Interferon titres were expressed as the dilution of plasma which would reduce the plaque count to 50 per cent of that of the controls and were calculated by probit analysis.

Preliminary studies showed that mice given 250 μg of endotoxin intravenously exhibited the usual pattern of peak interferon levels in the plasma 2 h later[1]; however, a second dose of endotoxin given 48 h later failed to produce circulating interferon. To further document and extend this observation, the following experiment was performed using challenge with NDV as well as endotoxin. A large group of mice was injected intravenously with 125 μg of endotoxin. Forty-eight hours later these animals were challenged intravenously with either a second dose of 125 μg of endotoxin or with 5×10^7 plaque-forming units of NDV. Control mice which had received no prior endotoxin were also challenged with endotoxin or NDV as described. Because of the different time patterns of appearance of interferon, blood samples were obtained at 2, 4 and 6 h in the case of endotoxin challenge and at 2, 6 and 12 h from animals injected with NDV. The results presented in Fig. 1 show that prior treatment with endotoxin completely eliminated the characteristic appearance of interferon which followed endotoxin inoculation of untreated mice. In the case of NDV challenge, mice treated 48 h previously with endotoxin showed a markedly depressed interferon titre compared to the untreated controls. The untreated mice showed significant interferon levels 2 h after injection of NDV. However, mice pretreated with endotoxin did not produce a detectable level of interferon until 6 h after stimulation. At 12 h after inoculation of NDV, the interferon titre in control mice was 1 : 2,500, while in mice pretreated with endotoxin the titre had reached only 1 : 140. It is of interest to note that, although the levels of circulating interferon in the two groups of mice were significantly different, the rate of interferon increase after 6 h was the same.

Another experiment was performed in which mice were injected with different amounts (50 or 250 μg) of endotoxin and challenged 48 h later with a large dose (1,000 μg) of endotoxin, *B. abortus*, or decreasing amounts of NDV. The results obtained are summarized in Table 1. It is

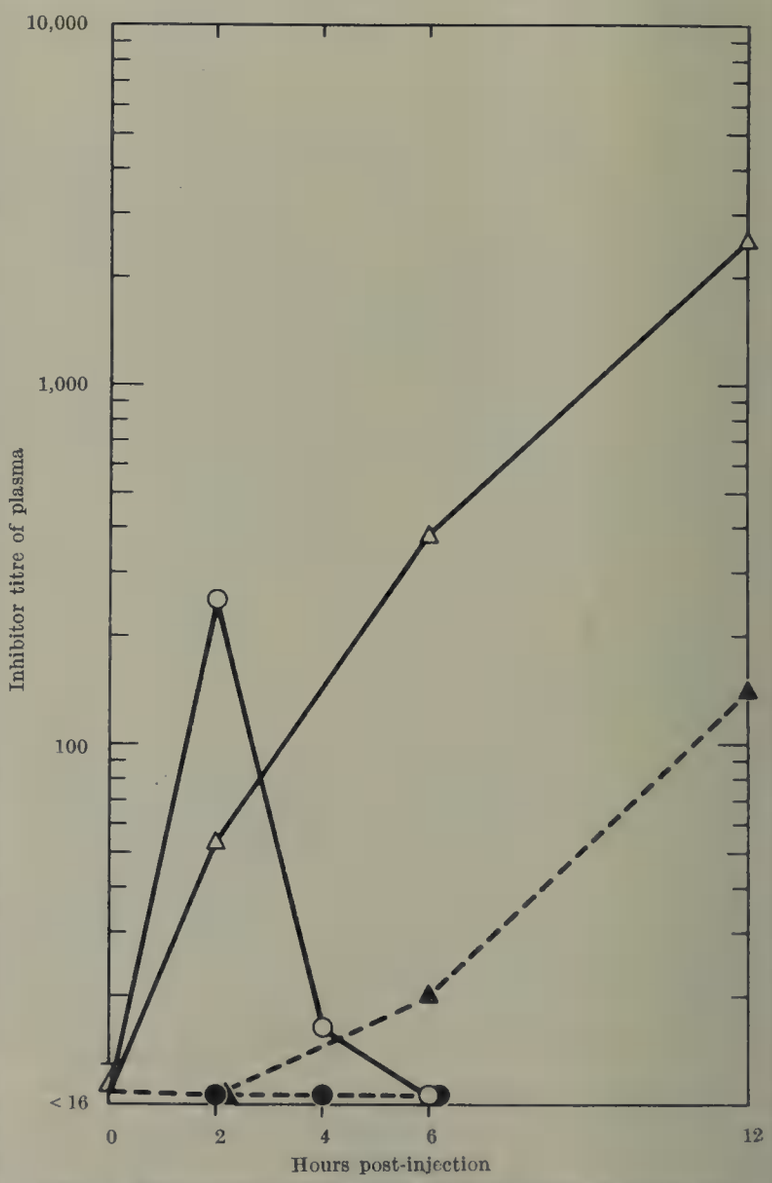

Fig. 1. Effect of prior injection with endotoxin on interferon titres of plasma pools from mice at different times after intravenous injection of endotoxin or Newcastle disease virus (NDV). Groups injected as follows: ○, no prior treatment, 125 μg endotoxin; ●, pretreatment with 125 μg endotoxin 48 h before 125 μg endotoxin; △, no prior treatment, 5×10^7 plaque-forming units of NDV; ▲, pretreatment with 125 μg endotoxin 48 h before 5×10^7 plaque-forming units of NDV

clear that both doses of endotoxin produced hyporeactivity although the higher dose (250 μg) was much more effective. In addition, decreased interferon responses were demonstrable in animals challenged with *B. abortus* as well as with endotoxin and NDV. It should be noted that no significant change in lethality of endotoxin was found in mice 48 h after the original dose of endotoxin.

The possibility was considered that circulating humoral factors present 48 h after endotoxin injection were responsible for the hyporeactive state. To test this in the case of endotoxin, 50 mice were injected intravenously with 250 μg of endotoxin; 48 h later, the survivors were bled and plasma collected and pooled. Before transferring this plasma pool to recipient mice, tests of the plasma pool showed that no residual interferon was detectable, eliminating the possibility of transfer of interferon to the recipients. Ten uninoculated mice received intraperitoneally 1 ml. of the donor plasma pool. Six hours later these mice and control mice were injected with 1,000 μg of endotoxin; 2 h later they were bled to determine the interferon content of their plasmas. Control mice had received plasma from untreated mice rather than from mice treated with endotoxin. No difference was noted in the interferon content of plasmas from the two groups of animals following the endotoxin challenge. These results tend to rule out the possibility of a circulating humoral factor which might mediate the lack of response to the second dose of endotoxin.

Table 1. Appearance of Interferon following Injection of Endotoxin, *B. abortus*, and NDV in Control Mice and in Mice injected 48 h previously with a Single Dose of *E. coli* Endotoxin

Challenge of mice at 48 h with:	Interferon titre of plasma of mice injected 48 h previously with: Saline (0·1 ml. i.v.)	50 μg endotoxin (i.v.)	250 μg endotoxin (i.v.)
Endotoxin (1,000 μg)	1,750*	45	< 16
B. abortus ($1·5 \times 10^9$ viable units)	560†	70	< 16
NDV ($4·1 \times 10^8$ P.F.U.)	1,400†	1,100	330
NDV ($4·1 \times 10^7$ P.F.U.)	860	350	200
NDV ($4·1 \times 10^6$ P.F.U.)	50	55	< 16
Saline (0·1 ml. i.v.)	< 16	—	—

* Mice challenged with endotoxin were bled 2 h after injection.
† Mice challenged with *B. abortus* or NDV were bled 10 h after injection.
The lot of endotoxin used in this experiment had an LD_{50} of 420 μg.

Previous experience using passively transferred anti-NDV chicken serum in mice showed that interferon did not appear following NDV injection into recipients with circulating antibodies (Table 2). To test for the presence of virus-neutralizing substances in mice which had been rendered hyporeactive to endotoxin and NDV, the following was done. Mice were injected with 125 μg of endotoxin (Fig. 1); 48 h later they were bled without challenge and their plasmas were tested for NDV-neutralizing activity by a plaque-reduction method using chick embryo fibroblast cultures. No NDV-neutralizing activity was found. This result eliminated antibody as the mediating factor in decreased reactivity to NDV.

Table 2. Effect of Passive Immunization of Mice with Anti-NDV Serum on Interferon Titres following Intravenous Injection of NDV

Treatment	Interferon titre of plasma at 4 h	12 h
Anti-NDV chicken serum†	< 16‡	< 64‡
Normal chicken serum*	256	> 4,096

* One ml. serum injected intraperitoneally 18 h prior to challenge with 5×10^7 plaque-forming units of NDV.
† Plasma from passively immunized mice prior to challenge with NDV showed a neutralizing titre of 1 : 1,250 by the plaque-inhibition method using chick embryo fibroblasts as host cells.
‡ Lowest dilution tested.

In order to determine whether or not prior injection of NDV would result in decreased interferon stimulation by endotoxin, a large group of mice was injected intravenously with 2×10^8 plaque-forming units of NDV. The titre of interferon in representative animals was determined using plasmas obtained 2, 6, 12, 24, 48 and 72 h after inoculation of virus. At 48, 72 and 144 h after

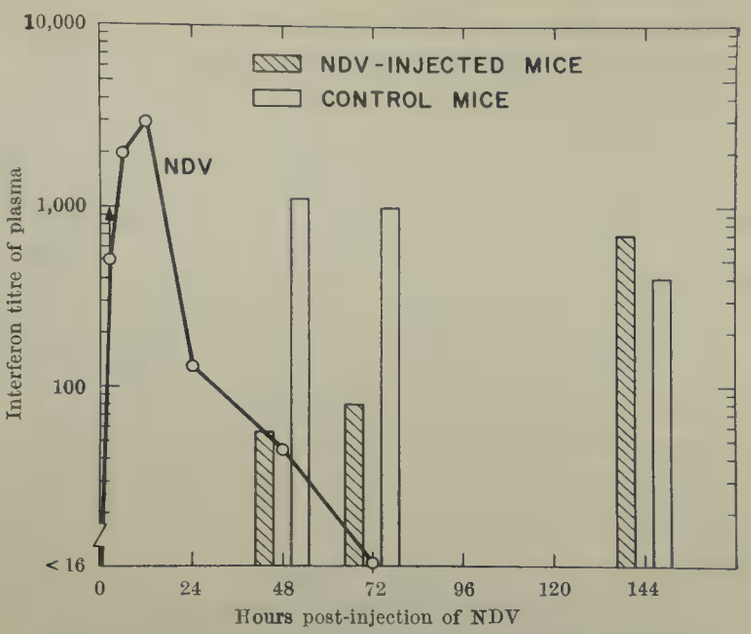

Fig. 2. Influence of prior injection of NDV on interferon response of mice to endotoxin. Interferon titres following injection of 2×10^8 plaque-forming units of NDV (○); a challenge dose of 500 μg of endotoxin was given at 48, 72, and 144 h and plasmas were collected 2 h later (see bars)

the single dose of NDV, virus-treated and control mice were injected intravenously with 500 μg of endotoxin; 2 h later these animals were bled and their plasmas tested for interferon. The results of this experiment, presented in Fig. 2, show that NDV effectively decreased interferon response to endotoxin; this decreased response to endotoxin did not disappear until between 72 and 144 h after injection of NDV. It should be noted that 48 h after virus injection there was a residual interferon titre of 1 : 45 in the plasma and no additional interferon was detected after endotoxin inoculation; in control mice the same dose of endotoxin produced a titre of 1 : 1,100. At 72 h after NDV inoculation, when no detectable residual interferon was present in the circulation, there was about 90 per cent inhibition of interferon appearance in virus-treated mice as compared with controls. By 144 h after NDV injection, full reactivity to endotoxin had returned. The preceding experiments show: (1) that the decreased appearance of interferon in hyporeactive animals is not limited to the material used to produce this state; (2) that no detectable humoral factor is responsible for the decreased interferon response induced by the injection of endotoxin or NDV.

Hyper-reactivity, the enhanced state of responsiveness to the lethal and pyrogenic effects of endotoxin, is known to occur in mice infected with attenuated tubercle bacilli[4]. Results are presented here to show that infection with *M. tuberculosis*, strain B.C.G., increases the reactivity of mice to endotoxin when measured by interferon response as well as by lethality. Mice were infected intravenously with 0·1 mg of a 10-day culture of B.C.G. grown in a modified Dubos–Middlebrook medium[5]. Fourteen days later, LD_{50} determinations with endotoxin were made using infected and un-infected mice. In addition, groups of infected and control mice received different doses of endotoxin, and interferon determinations were made with plasmas

Table 3. Influence of Hyper-reactivity following *BCG* Infection on Interferon Titres following Different Doses of *E. coli* Endotoxin

Group	LD_{50} of endotoxin	Amount of endotoxin used for interferon stimulation (μg)	(LD_{50})	Interferon titre of plasma at 2 h
B.C.G.-infected mice (infected 14 days prior to testing)	23 μg	32	1·4	> 4,096
		3·2	0·14	2,300
		0·32	0·014	120
		0·032	0·0014	< 16
Un-infected mice	540 μg	500	0·9	> 2,048
		50	0·09	200
		5	0·009	200
		0·5	0·0009	30

obtained from bleedings 2 h later. The results are shown in Table 3. The mice infected with B.C.G. showed a 23-fold increase in sensitivity to the lethal effects of endotoxin. Associated with this, infected mice demonstrated a markedly enhanced ability to produce interferon following decreasing doses of endotoxin. For example, in infected mice 3·2 μg of endotoxin produced the same interferon titre as 500 μg in un-infected animals. Additional experiments, in which the degree of hyper-reactivity varied depending on the duration of infection, confirmed the correlation between heightened interferon-levels and increased sensitivity to the lethal effects of endotoxin.

The appearance of interferon in the circulation following the injection of *B. abortus* and NDV was tested in B.C.G.-infected mice which were hyper-reactive to the lethal and interferon-stimulating effects of endotoxin. No significant differences were found in the responses of infected and control mice to *B. abortus* and NDV over a wide dose-range.

The experiments which have been described indicate that the appearance of interferon in the circulation of mice can be affected by procedures which alter responsiveness to the lethal and pyrogenic effects of endotoxin. Hypo-reactivity produced with endotoxin inhibited responsiveness, as measured by interferon-levels, to endotoxin, *B. abortus*, and NDV; moreover, prior inoculation of NDV markedly reduced interferon stimulation by endotoxin. These results indicate the likelihood that the same cells, probably phagocytic, are involved in the release of interferon following both types of stimulation, despite the different time patterns of appearance of interferon in the circulation[1].

In the case of hyper-reactivity to endotoxin elicited by infection with tubercle bacilli, it is apparent that the increased lethality of endotoxin is accompanied by an increased capacity of small doses of endotoxin to elicit interferon. The lack of a parallel increase in response to different doses of *B. abortus* or NDV in B.C.G.-infected mice is not explained by the results presented here and is being investigated further.

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ALLOGENEIC INHIBITION OF TUMOUR CELLS BY *IN VITRO* CONTACT WITH CELLS CONTAINING FOREIGN *H*-2 ANTIGENS

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HOMOZYGOUS tumour cells grow in a higher frequency and after a shorter latency period when small cell numbers are transplanted to syngeneic hosts than to 'semi-syngeneic' F_1 hybrids[1–3]. This phenomenon has been designated by the term 'syngeneic preference' in relation to the homozygous host and 'allogeneic inhibition' with reference to the F_1 hybrid host[4].

Allogeneic inhibition could not be explained by a host-versus-graft reaction against hypothetical antigens which might have been present in the homozygous cells and hosts but absent from the F_1 hybrid hosts, since pre-irradiation of the F_1 animals did not abolish the difference and since pretreatment of the F_1 hybrids prior to grafting with normal or tumour cells of the homozygous strain did not influence allogeneic inhibition[5]. Tissue culture experiments showed that cell extracts or homogenates containing foreign histocompatibility antigens inhibited the growth of explanted target cells, suggesting that allogeneic inhibition *in vivo* might be due to the contact between the grafted tumour cells and the foreign isoantigens of the host[5,6]. This hypothesis was subjected to further test in the present article. Neutralization experiments were made in which lymphoma cells were exposed *in vitro* to spleen or tumour cells, containing foreign *H*-2 antigens, and their outgrowth in syngeneic hosts was measured after subsequent implantation. Furthermore, tissue culture experiments were performed, in which explanted tumour cells were followed for the development of cytopathic changes after exposure to lymphoma cells, containing *H*-2 antigens of the syngeneic or allogeneic type.

Neutralization experiments. Four isoantigenic variant sub-lines were used, all derived from the *LNSF* lymphoma (of *A/Sn* × *A.SW* F_1 hybrid origin). Three lines, designated *LNSFSC*, *LNSFSD* and *LNSFSE*, had been selected from *LNSF* for compatibility with the *A.SW* parental strain, and permanently lacked detectable *H*-2 antigens specific for the *A* strain[5]. One line, *LNSFAD*, had been selected from *LNSF* for compatibility with strain *A*. It lacked detectable *H*-2 antigens that were specific to the *A.SW* strain[5]. The variant lines were propagated by serial transfer in syngeneic mice (*A.SW* or *A*, respectively) during 1–10 passages.

The technique used for the neutralization tests was a modification of one described by Klein and Sjögren[7]. *A.SW*-compatible variant tumours (*LNSFSC*, *LNSFSD* or *LNSFSE*) were gathered from syngeneic (*A.SW*) mice and cell suspensions were prepared mechanically and diluted in balanced salt solution to give a concentration of 10^4 or 10^5 cells per ml. Similar suspensions were prepared from the spleens of adult, untreated mice of various genotypes (*A*, *A.BY*, *A.SW*, *CBA*, *A.SW* × *A* F_1, *A.SW* × *A.BY* F_1, *A.SW* × *CBA* F_1). Among these strains *A*, *A.SW* and *A.BY* are congeneic resistant lines, differing at the *H*-2 locus[8]. Within each experiment spleens were taken from mice of the same sex (mostly virgin females), weight and age. After washing, the spleen cells were diluted to a final concentration of 10^8 blue unstained cells/ml. In half the experiments they were irradiated *in vitro* immediately after preparation of the cell suspension with an X-ray dose of 1,000 r. (or in two experiments up to 10,000 r.). Tumour and spleen cells were mixed in equal volumes and Wellcome phytohaem-agglutinin (PHA) added in a final concentration of 1 : 100 to establish a close contact between the tumour and spleen cells[9,10]. The mixtures were thoroughly shaken and incubated for 45 min at 37° C. Afterwards they were inoculated subcutaneously to adult *A.SW* mice to yield a total number of 10^3 or 10^4 tumour cells inoculated per mouse. The recipient mice had been irradiated with 350 r. immediately before inoculation in order to decrease their own immunological reactivity. They were inspected 2–3 times weekly for tumour development, three diameters

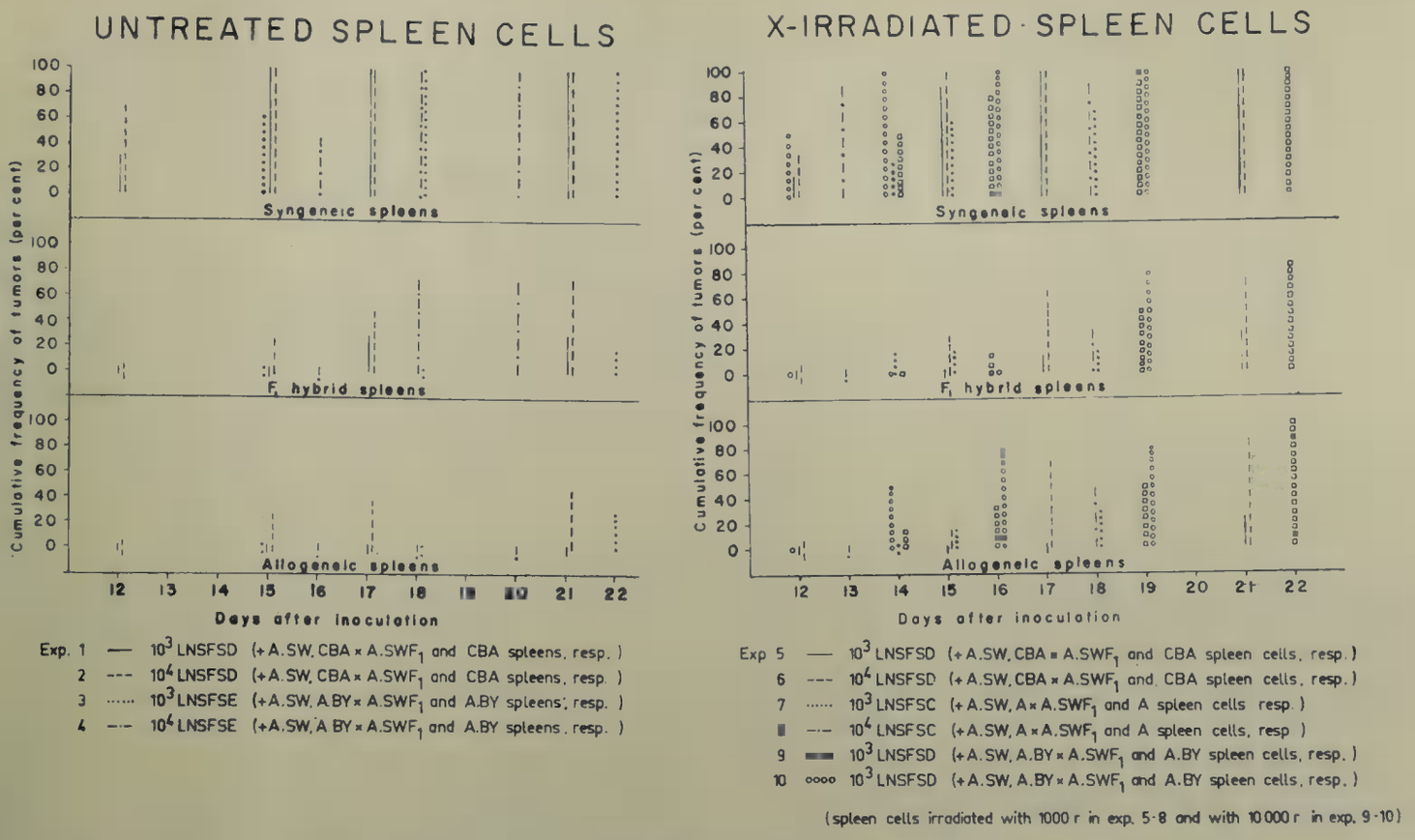
UNTREATED SPLEEN CELLS
X-IRRADIATED SPLEEN CELLS
Cumulative frequency of tumors (per cent)
Syngeneic spleens
F1 hybrid spleens
Allogeneic spleens
Days after inoculation
Exp. 1 — 10³ LNSFSD (+A.SW, CBA × A.SWF1 and CBA spleens, resp.)
2 --- 10⁴ LNSFSD (+A.SW, CBA × A.SWF1 and CBA spleens, resp.)
3 ..... 10³ LNSFSE (+A.SW, A.BY × A.SWF1 and A.BY spleens, resp.)
4 -.- 10⁴ LNSFSE (+A.SW, A.BY × A.SWF1 and A.BY spleens, resp.)
Exp 5 — 10³ LNSFSD (+A.SW, CBA × A.SWF1 and CBA spleen cells, resp.)
6 --- 10⁴ LNSFSD (+A.SW, CBA × A.SWF1 and CBA spleen cells, resp.)
7 ..... 10³ LNSFSC (+A.SW, A × A.SWF1 and A spleen cells resp.)
-.- 10⁴ LNSFSC (+A.SW, A × A.SWF1 and A spleen cells, resp.)
9 ▬ 10³ LNSFSD (+A.SW, A.BY × A.SWF1 and A.BY spleen cells, resp.)
10 oooo 10³ LNSFSD (+A.SW, A.BY × A.SWF1 and A.BY spleen cells, resp.)
(spleen cells irradiated with 1000 r in exp. 5-8 and with 10 000 r in exp. 9-10)

Fig. 1. Neutralization of lymphoma cell outgrowth in syngeneic mice, pre-irradiated with 350 r. after exposure to untreated or X-irradiated spleen cells and phytohaemagglutinin *in vitro*. In each group of experiments 6–8 mice were inoculated

of any tumours appearing being measured and the geometric means calculated.

Fig. 1 shows that allogeneic and F_1 hybrid spleen cells inhibited the outgrowth of the lymphoma lines, as compared to syngeneic spleen cells. When unirradiated spleen cells were added, F_1 hybrid cells were slightly less effective than allogeneic cells. Pre-irradiation of the spleen cells minimized this difference by decreasing the inhibitory effect of the allogeneic cells but scarcely affecting the effect of F_1 hybrid cells.

The demonstration of allogeneic inhibition after contact of tumour cells with spleen cells, carrying foreign *H*-2 isoantigens, led to experiments where the spleen cells had been replaced with tumour cells. This was done in order to test the possibility that neutralization as observed after addition of spleen cells was due to an immunological reaction of the added cells not sensitive to X-irradiation. Experiments were performed in a similar manner as when spleen cells were added. Two variant lines derived from *LNSF* were used, namely, *LNSFAD*, compatible with strain *A* and lacking *H*-2 antigens of *A.SW* origin, and *LNSFSE*, compatible with *A.SW* and lacking *H*-2 antigens of *A* origin. In each experiment, suspensions containing 10^4 or 10^5 untreated lymphoma cells of one type were mixed with an equal volume containing 10^8 lymphoma cells of the other type which had been rendered incapable of multiplication by X-irradiation with 15,000 r.; in controls, irradiated lymphoma cells of the syngeneic type were added. After incubation in the presence of PHA the mixtures were inoculated in mice, syngeneic to the untreated cells, 6–8 mice being inoculated in each experimental group. In part of the experiments, the irradiated cells were ultrasonically disintegrated before they were added to the untreated lymphoma cells.

A total of four neutralization experiments were performed with added lymphoma cells (or cell extracts) and gave similar results, as exemplified by the experiment presented in Fig. 2. It appears that exposure of *LNSFAD* cells (*A*-compatible) to *LNSFSE* cells (*A.SW*-compatible)

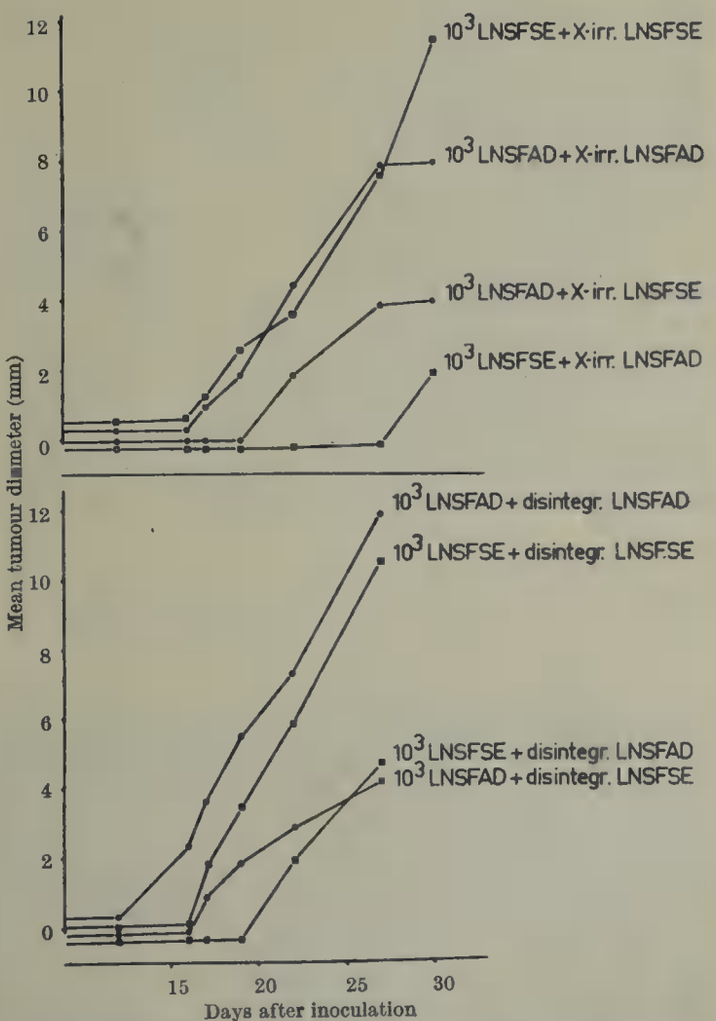
Mean tumour diameter (mm)
10³ LNSFSE + X-irr. LNSFSE
10³ LNSFAD + X-irr. LNSFAD
10³ LNSFAD + X-irr. LNSFSE
10³ LNSFSE + X-irr. LNSFAD
10³ LNSFAD + disintegr. LNSFAD
10³ LNSFSE + disintegr. LNSFSE
10³ LNSFSE + disintegr. LNSFAD
10³ LNSFAD + disintegr. LNSFSE
Days after inoculation

Fig. 2. Neutralization of outgrowth of lymphoma variants *LNSFAD* and *LNSFSE* in syngeneic (*A* and *A.SW*, resp.) mice after exposure to X-irradiated (15,000 r.) intact and ultrasonically disintegrated *LNSFAD* and *LNSFSE* cells. In each group of experiments 6 mice were inoculated

Table 1. Records of Cultures exposed to Lymphoma Variant Cells containing *H*-2 Isoantigens of the Syngeneic or Allogeneic Type

Exp. No.	Explanted tumour		Days after addit. of lymphoma cells	Records of cultures given*						Ratio of records with syng : allog. ly. c.‡		
				LNSFAD (*A*-comp.)†			*LNSFSD* (*A.SW*-comp.)†					
	Designation	Strain of origin		2×10^7	10^7	5×10^6	2×10^7	10^7	5×10^6	2×10^7	10^7	5×10^6
1	*S1A*	*A*	4		3·3	3·7		2·0	3·0		1·7	1·2
			5		2·7	3·3		1·3	2·3		2·1	1·4
			7		2·3	3·0		0·3	2·0		7·7	1·5
	MWE	*A.SW*	4		1·0	1·3		2·3	3·7		2·3	2·8
			5		1·0	1·0		2·3	3·0		2·3	3·0
			9		0·3	0·7		5·0	5·0		16·6	7·1
2	*S1A*	*A*	3		4·0	4·0		1·0	2·0		4·0	2·0
			6		4·0	4·3		1·0	2·0		4·0	2·2
	MWE	*A.SW*	2		2·0	3·0		3·0	3·0		1·5	1·0
			3		2·0	2·3		2·6	4·0		1·3	1·7
			6		2·0	3·0		3·0	4·0		1·5	1·3
3	*S1A*	*A*	3		2·7	3·0		1·5	2·5		1·8	1·2
			4			4·0			2·5			1·6
	MWE	*A.SW*	3	2·0	4·0		3·5	4·0		1·8	1·0	
			4	2·0	4·0		4·0	5·0		2·0	1·3	
4	*YAA-C1-C3*	*A*	3	4·3	4·0	5·0	0·5	4·0	4·8	8·6	1·0	1·0
			6	4·0	4·0	5·0	0·5	0·5	3·5	8·0	8·0	1·4
5	*YAA-C1-C3*	*A*	3	2·5	3·0		1·0	3·0		2·5	1·0	
			5	2·0	4·0		1·0	3·0		2·0	1·3	
			10	3·0	5·0		1·0	4·0		3·0	1·3	

* Cultures were observed for development of cytopathic changes after addition of 2×10^7, 10^7 and 5×10^6 syngeneic or allogeneic lymphoma cells and compared with untreated control cultures, consisting of monolayers of apparently healthy cells. They were recorded as follows: 5, treated cultures better than untreated controls; 4, no difference between treated cultures and untreated controls; 3, approximately 25 per cent of cells destroyed; 2, approximately 50 per cent of cells destroyed; 1, only a few cells surviving; 0, all cells destroyed. The Table shows the mean of records given to a minimum of 3 Petri dishes which had been exposed to the same treatment.

† *LNSFAD* and *LNSFSD* cells were gathered from $A \times A.SW$ F_1.

‡ Ratios were calculated between records with added syngeneic as compared to allogeneic lymphoma cells (allog. ly. c.).

inhibited the outgrowth of *LNSFAD* and that growth of *LNSFSE* was inhibited after contact with intact or disintegrated *LNSFAD* cells.

The neutralization experiments demonstrate that differences between homozygous and F_1 hybrid mice with regard to general host factors such as endocrine balance, immunological reactivity or hybrid vigour cannot account for allogeneic inhibition. The finding that tumour outgrowth was inhibited, both by pre-irradiated F_1 hybrid spleen cells and by intact or disintegrated lymphoma cells carrying foreign *H*-2 antigens, gives increased weight to the hypothesis that allogeneic inhibition depends on a close contact between cells carrying different histocompatibility antigens, and thus having a different surface structure[6]. This is also strengthened by (unpublished) experiments where the inhibitory effect of added allogeneic or F_1 hybrid cells was abolished if their *H*-2 antigens were specifically covered with antibodies.

Tissue culture experiments. *In vitro* experiments were performed in order to definitely exclude all influence of the host animal on neutralization of tumour growth as observed after addition of cells carrying foreign *H*-2 antigens. Transplanted tumours of *A* or *A.SW* origin were explanted and the cultures were exposed to added lymphoma cells from *A*- or *A.SW*-compatible variant sub-lines, selected from *LNSF*. Three kinds of tumour cells were explanted: *S1A*, *MWE* and *YAA-C1-C3*. *S1A* is a mammary carcinoma of *A* origin, *MWE* a sarcoma of *A.SW* origin and *YAA-C1-C3* a cloned lymphoma of *A* origin[11]. Explantation was made into 50-mm 'Falcon' plastic Petri dishes, each Petri dish being seeded with 3×10^6 tumour cells. About 24 h after seeding, the culture medium was decanted and suspended lymphoma variant cells of the *LNSFAD* (*A*-compatible) or *LNSFSD* (*A.SW*-compatible) lines were added in 0·2-ml. volumes to each Petri dish, the lymphoma suspensions containing between $2{\cdot}5 \times 10^7$ and 10^8 cells per ml. In addition, 0·2 ml. PHA, diluted 1 : 20, was added. After 45 min incubation at 37° C, 4-ml. culture medium, containing 20 per cent heat-inactivated calf serum in Earle's solution, was added to each Petri dish, and the dishes were incubated in a 5 per cent carbon dioxide–air atmosphere. The Petri dishes were followed for appearance of cytopathic changes, which were recorded as stated in the footnote to Table 1. A minimum of three Petri dishes were used for each kind of treatment. All dishes were given coded numbers at the time of addition of the lymphoma cells, the code being unknown to the persons observing the cultures.

Findings are presented in Table 1. Addition of lymphoma cells containing *H*-2 antigens, allogeneic to the explants, consistently produced cytopathic changes in the explanted cells with very small variation between individual Petri dishes, exposed to the same type and number of lymphoma cells. The fact that both lymphoma lines (*LNSFAD* and *LNSFSD*) were gathered from $A \times A.SW$ F_1 hybrid mice and tested on tumours for which they were syngeneic and allogeneic, respectively, excludes influence of normal stroma cells of host origin on the results. It follows that the cytopathic changes detected after exposure of explanted cells to allogeneic lymphoma variants are scarcely a consequence of immune reactions of the added cells, unless the assumption is made that the lymphoma cells are capable of immunological reactivity; the fact that neither pre-irradiation nor ultrasonic disintegration of the lymphoma cells abrogated their ability to neutralize tumour growth *in vivo* excludes the latter possibility. Both neutralization and tissue culture experiments therefore strongly support the hypothesis that allogeneic inhibition of tumour growth is a consequence of target cell contact with foreign *H*-2 isoantigens[6]. It is suggested that allogeneic inhibition may function as a surveillance mechanism, capable of eliminating cellular variants, the (*H*-2) antigenicity of which differs from the general pattern within a given tissue, without necessary engagement of immunologically competent cells.

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SPECIFIC UPTAKE IN CORPORA LUTEA OF A NON-STEROID SUBSTANCE WITH ANTI-GESTAGENIC PROPERTIES

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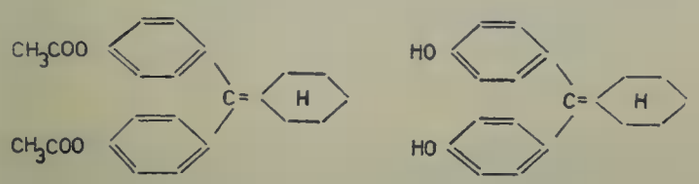
CERTAIN unsymmetrical diphenylalkenes have been found to possess the ability to interfere with the vaginal oestrous reaction induced by oestradiol benzoate[1,2]. Among these substances, *bis*(*p*-hydroxyphenyl)-*cyclo*-hexylidenemethane (compound '*F*6060', Fig. 1) has an oestrogenic activity only about 1/1,000 that of oestradiol benzoate.



Compound '*F*6066' Compound '*F*6060'

Fig. 1

In clinical investigations it was found that the acetylated form of the compound (Compound '*F*6066') had an effect on prostatic cancer similar to conventional oestrogens but without the feminizing side-effects[3]. In postmenopausal women a depression of the output of oestradiol-17β and changes in the relation between excreted FSH and LH were found[4].

By determining the body distribution of the compound it was hoped to gain further information regarding their site of action. The substances were labelled with ^{14}C at the α-carbon[5]. The specific activity was about 10 μc./mg.

The compounds, which were synthesized by Ferrosan, Ltd., Sweden, were dissolved in absolute alcohol and mixed with water containing 'Tween 80' (5 per cent).

Female mice, non-pregnant and pregnant in a late stage of gestation, were used. A dose of 0·8 mg corresponding to 8 μc. was given intravenously. The mice were killed by rapid freezing at the following intervals after injection: 5 min, 20 min, 1 h, 4 h and 24 h.

20-μ sections at various levels through the whole animal were taken at −10° C. The sections were freeze-dried and pressed against Gevaert 'Structurix *D* 7' X-ray film[6].

The autoradiograms showed a remarkably strong and selective accumulation of radioactivity in corpora lutea after only 5 min (Fig. 2). This accumulation was still prominent after 20 min (Fig. 3) and then decreased successively but was still apparent after 24 h (Fig. 4). It was most pronounced in the pregnant mice. In non-pregnant mice the accumulation in corpora lutea varied, probably owing to different functional states.

A selective accumulation was also noticed in part of the placenta. The activity was confined to the visceral yolk sac epithelium and was seen from 1 to 24 h (Fig. 4) with a peak at 4 h. From this time and during the whole observation period it showed the highest activity in the body. A detailed description of the autoradiographic results will be published elsewhere.

The influence of '*F*6066' on early pregnancy was tested in rats and rabbits. Groups of six female rats were caged together with males, two females to one male. On the first and second day the females were injected subcutaneously with an oil solution of '*F*6066'. The injections were then repeated three times at weekly intervals. If pregnancy was observed the animals were killed just before parturition. On the day of the last injection the males were removed and 3 weeks later all females were killed for examination of possible pregnancy and number of living foetuses. The results are presented in Table 1. The number of living foetuses decreased with increased dose. The highest dose used was < 1·5 per cent of the acute LD_{50}.

Table 1. ANTI-FERTILITY IN RATS

	No. of pregnancies per 6 animals	No. of fully developed foetuses per 6 animals
Olive oil	5	51
1 mg/kg/day '*F*6066'	4	41
5 mg/kg '*F*6066'	1	11
25 mg/kg '*F*6066'	0	0

In a modified Clauberg test, immature female rabbits (600–800 g) were primed with 0·5 μg oestradiol benzoate for 7 days. From days 8 to 14 they were all injected with 0·2 mg progesterone in olive oil, subcutaneously, and half of them also received '*F*6066' simultaneously, on days 8–14 or days 13–14.

In the Clauberg test, injections of oestrogens produce a proliferation of the endometrium. Progesterone injections

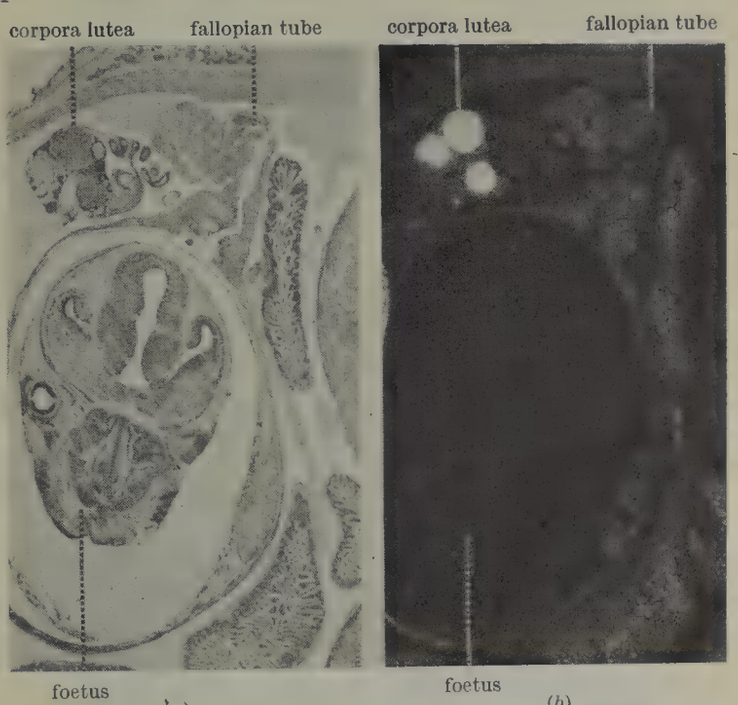


Fig. 2. Autoradiogram (*b*) showing the distribution of ^{14}C-'*F*6060' in ovary, fallopian tube and pregnant uterus 5 min after intravenous injection. Corresponding tissue section (*a*) is stained by haemalum-eosin. Note the very high concentration in corpora lutea. No activity in the foetus

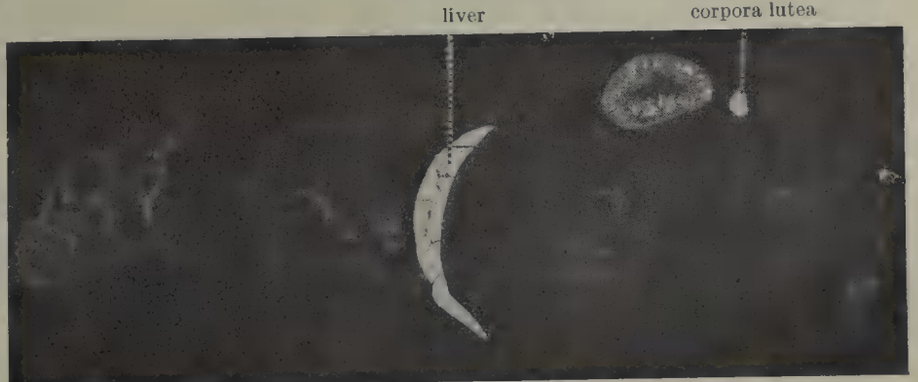


Fig. 3. Whole-body autoradiogram from a pregnant mouse 20 min after intravenous injection of ^{14}C-'*F*6066'. Note high radioactivity in corpora lutea and liver

transform this to a secretory endometrium. Simultaneous administration of 1 mg '*F*6066' from days 8 to 14 prevented the development of the secretory endometrium. If 5 mg '*F*6066' was injected on days 13 and 14, when the secretory phase was established, a regression could be seen (Figs. 5 and 6).

The present investigation has shown that the substances have: (1) a selective and high accumulation in corpora lutea; (2) an anti-fertility activity in rats; and (3) an anti-progesterone effect in the Clauberg test.

The strong affinity for corpora lutea is unique and may indicate an interference with progesterone production. The lack of accumulation in some corpora lutea may indicate that the affinity is not related to the cell-type as such but to the intensity of hormone production.

On the other hand, the Clauberg test shows that the substances interfere with the effect of injected progesterone, that is, have a peripheral effect on a target organ. It is known that natural oestrogens and stilboestrol have similar properties. Thus the observed effect may be due to the weak oestrogenic activity. In the anti-fertility tests, however, the autopsies after administration of '*F*6066' did not show the large volume of sanguinary fluid in the uterine cavity that is often seen after stilboestrol-induced abortion.

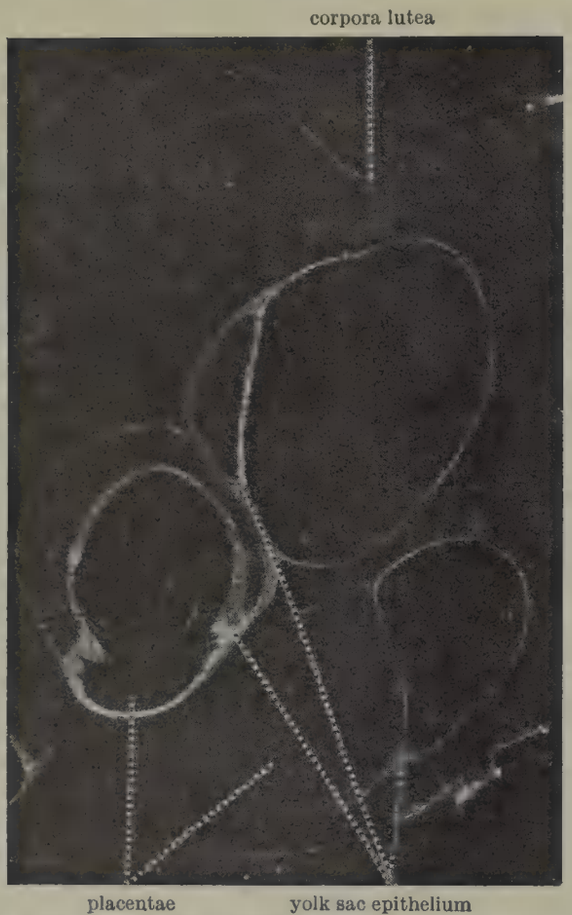


Fig. 4. Autoradiogram showing the distribution of ^{14}C-'*F*6060' in a pregnant mouse 24 h after intravenous injection. Note high radioactivity in the visceral yolk sac epithelium. Radioactivity is seen in 5 corpora lutea. Three placentae and foetuses show no radioactivity

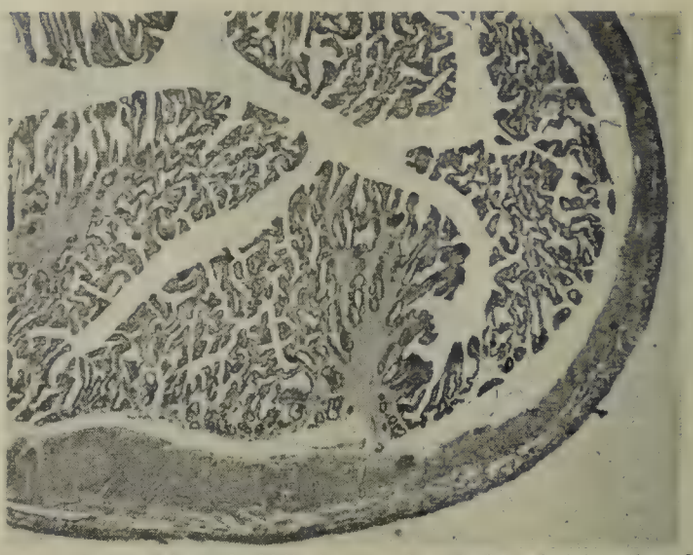


Fig. 5. Endometrium of rabbit uterus on day 15 after administration of 0·5 μg oestradiol benzoate on days 1–7, and 0·2 mg progesterone on days 8–14

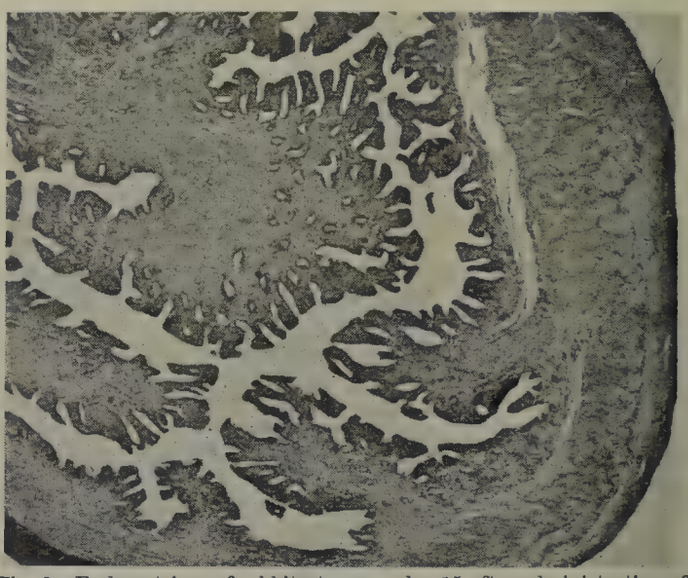


Fig. 6. Endometrium of rabbit uterus on day 15 after administration of 0·5 μg oestradiol benzoate on days 1–7, 0·2 mg progesterone on days 8–14 and 5·0 mg '*F*6066' on days 13 and 14

The observations made may indicate interference with gestagen production as well as an inhibition of the gestagen effect on target organs. The accumulation found in the yolk-sac placenta may be explained as a foetal excretory event, but interference with progesterone synthesis may also be considered.

Laboratory equipment used in this investigation was kindly supplied by Ragnar och Torsten Söderbergs stiftelser.

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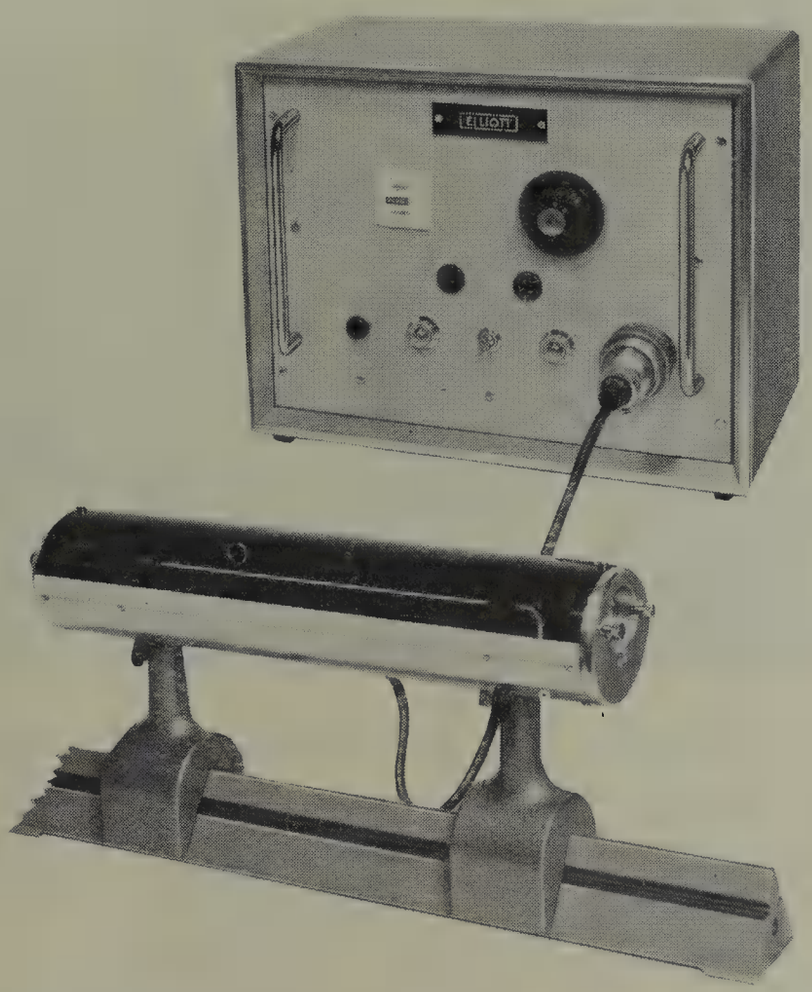

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assessed if a mosquito penetrates the skin of a host and salivates, but does not engorge. On the other hand, the method of direct visual observation is laborious, uncertain, and in many cases impractical. Since salivation is the vector by which mosquito-borne disease is disseminated, the measure of repellency of a compound by the degree of engorgement is seriously limited.

Through a new assay procedure recently developed in our laboratories, the various phases of the mosquito bite from the initial penetration of the mouth-parts to final withdrawal can be detected electronically. It was reasoned that the contact between a mosquito and its host could be used to form an integral part of a series electrical circuit, where the insertion and withdrawal of the mosquito's mouth-parts in relation to the host could be used as the making and breaking switch in the circuit.

Since it is impracticable to connect a wire directly to a mosquito, a 50- or 100-mesh bronze screen is used to separate the mosquito from its host. The screen covers a 1-pint round ice-cream carton (or glass container if simultaneous visual observation is desired). Female *Aedes aegypti* L. are placed into the container. The screen is connected by a soldered lead to resistances totalling 16 MΩ, and then to the negative pole of a 1·35-V standard mercury cell (Mallory mercury battery, *RM*-1-*R*). The positive pole of the battery is connected to the input of a suitable recording instrument. The recorder is a Sanborn model 320 two-channel general-purpose recorder (Sanborn Co., Waltham, Massachusetts).

The circuit passes through the recorder and is carried by another lead from the recorder to the tail of an anaesthetized mouse (the host), where it is embedded via a hypodermic needle soldered on to the lead. The mouse is placed on top of the bronze screen, belly down. Contact of the mouse's paws with the screen is carefully avoided. The hair on the mouse's body is sufficient to insulate the mouse from the screen, and no current flows. The entire circuit is thus connected in series (Fig. 1). The wiring diagram of the box which contains the resistors and the battery is shown in Fig. 2.

When a mosquito bites the mouse, the mosquito holds on to the wire screen with its legs and passes its proboscis through the spaces of the mesh and into the mouse. When the skin of the mouse is penetrated, the circuit between the mouse and the screen is completed through the mosquito. The resulting flow of current is registered by a displacement of the writing stylus of the recorder. The current flows continuously so long as the mosquito is completing the circuit, and the stylus is displaced from the base line (point of no current flow) on the recorder chart paper for the entire time the mosquito is biting. When the proboscis is withdrawn, the circuit is broken. The writing stylus immediately returns to the base line, indicating that virtually infinite resistance has been re-established between the mouse and the bronze mesh.

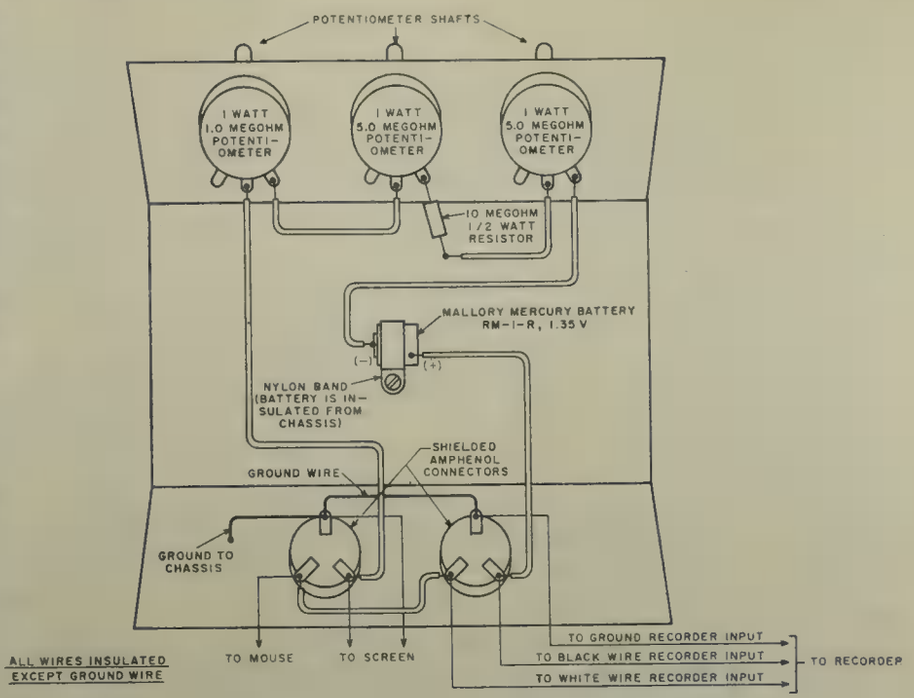

Fig. 2. Wiring diagram of resistor-battery box

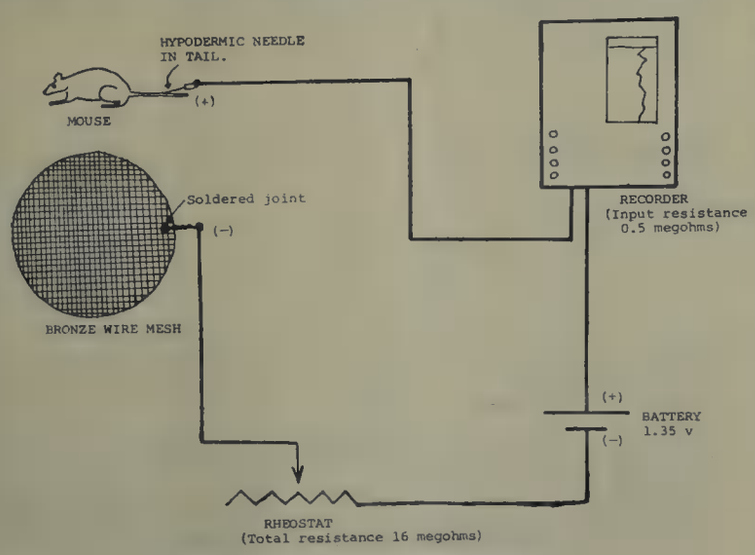

Fig. 1. Circuit diagram of apparatus

The question of whether the flow of an electric current could change a mosquito's behaviour or create artefacts in the system was considered. A calculation taking into account the total resistance of the circuit and the total voltage available showed that a maximum of $8{\cdot}2 \times 10^{-8}$ amp is available in the circuit, assuming no resistance between the mouse and the mesh. The average displacement of the stylus is 10 mm from the base line when a single mosquito bites a mouse through the screen. In calibrating the recorder (which was always set at its highest sensitivity level of 0·5 mV/mm and maximum gain), it was observed that a displacement of 3·4 mm from the base line is equivalent to 1 mV. A displacement of 10 mm (equivalent to an average single mosquito bite), therefore, indicates a voltage drop of 2·94 mV across the recorder.

From the known input resistance of the recorder (0·5 MΩ), and the measured voltage drop across the recorder when a mosquito bites, it can be shown that approximately $6{\cdot}0 \times 10^{-9}$ amp flows through a mosquito at the time of the bite. This is probably an over-estimation, since the resistance of the mosquito is not taken into account. This is an extremely small quantity of electricity, and is probably no more than the static electrical charge that the insect attains during free flight. We therefore consider that no artefacts that might affect a mosquito's activities are introduced by this flow of electrical current. We could not detect changes in a mosquito's behaviour even when all resistances were removed from the circuit. A value of 16 MΩ of resistance between the mesh and the battery seemed to give the most uniform recordings.

The question of whether biting was the only activity that could cause a departure from the base line in the recording was also considered. When male *Aedes aegypti* L. mosquitoes or female mosquitoes whose proboscises were amputated were placed in the mesh-covered container, no displacement from the base line was observed at any time. We therefore conclude that it is only the biting female mosquito which causes displacement.

A visual observation and an electronic recording were made simultaneously during the course of a single mosquito bite. The patterns recorded during the observed

probing activities of the mosquito were generally irregular (left side of Fig. 3). When the mosquito was observed to be in the biting attitude, the chart recording was much more regular (right side of Fig. 3). Engorgement was observed during the more regular phase of the recording. When the mosquito was fully engorged after about 1·5 min the proboscis was withdrawn from the mouse and the stylus immediately returned to the base line on the chart recording (Fig. 4).

When about 50 mosquitoes were placed in the container and permitted to bite simultaneously, the pattern was very irregular (Fig. 5). The departure from the base line was greater than seen with a single mosquito bite. This greater departure is expected, since the resistance between the mouse and the mesh during the biting of a group of mosquitoes is less than during the biting of a single mosquito. However, proportionality of stylus displacement could not be detected for the bites of succeeding mosquitoes. The first mosquito that bites changes the resistance of the system from an infinite to a finite value. Subsequent bites do not cause as large a change, and proportionality should therefore not be expected. In effect, when a group of mosquitoes bite, a number of miniature parallel circuits are set up between the mouse and the mesh. Assuming the resistances of all the mosquitoes are equal, each biting mosquito should draw the same quantity of current. In terms of amps, then, proportionality and additivity might be expected as the number of parallel units (biting mosquitoes) in the circuit increases. We are at present examining methods to quantify the apparatus so that the number of insects biting simultaneously can be ascertained.

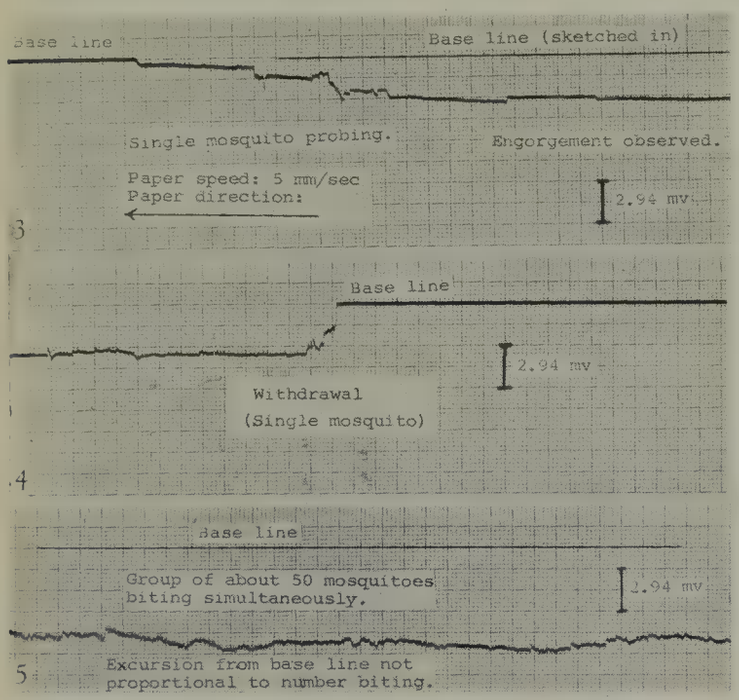

Fig. 3. (Top). Probing (left) and engorgement (right); single mosquito

Fig. 4. (Middle). Withdrawal of mosquito from mouse and return of stylus to original base line. Paper speed: 5 mm/sec. Paper direction: right to left

Fig. 5. (Bottom). Many mosquitoes biting mouse simultaneously. Paper speed: 5 mm/sec. Paper direction: right to left

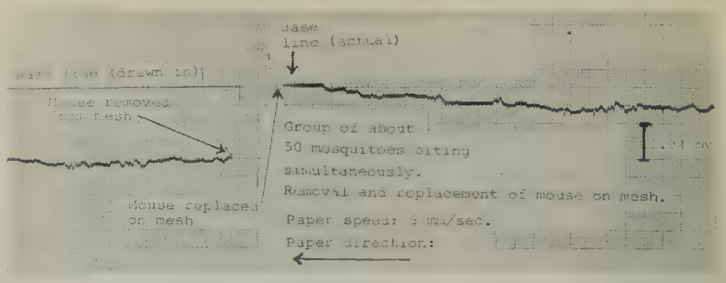
Base line (actual)
Mouse removed from mesh
Group of about 50 mosquitoes biting simultaneously.
Removal and replacement of mouse on mesh.
Mouse replaced on mesh
Paper speed: 5 mm/sec.
Paper direction:

Fig. 6. Removal and replacement of mouse on mesh during biting by a group of mosquitoes

During the recording of a group of mosquitoes biting simultaneously, the mouse was removed from the mesh, causing all the proboscises to be withdrawn from the mouse, and then replaced. Fig. 6 shows that, after the mouse was replaced on the mesh, the base line reverted to its original position. It was displaced again about 1·5 sec later when the mosquitoes resumed their attack on the mouse.

By changing the mesh size of the screen or the host animal, the apparatus can be adapted to determining the biting activities of many species of biting and blood-sucking insects, and the attractant, repellent and insecticidal properties of various compounds can be determined. By allowing the recorder to run overnight, or for a longer period of time, the duration of effective activity of a compound can be automatically monitored. The recorder is also capable of portable operation with a battery converter and can therefore probably be adapted for operation under actual field conditions.

This work was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract *DA–49–193–MD–2281*.

Note added in proof. A paper by McLean and Kinsey (*Nature*, **202**, 1358; 1964) describing a technique for recording electronically aphid feeding and salivation has recently come to our attention. Though similar in concept, the instrumentation, electrical circuitry, applications, and sensitivities of these techniques are different.

[1] Gordon, R. M., and Lumsden, W. H. R., *Ann. Trop. Med.*, **33**, 259 (1939).
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SYNTHESIS OF RIBOSOMAL RNA IN SYNCHRONIZED HeLa CELLS

By Prof. MATTHEW D. SCHARFF and Dr. ELLIOTT ROBBINS
Departments of Cell Biology, Medicine, Neurology and Microbiology, Albert Einstein College of Medicine, Bronx, New York

WHILE the synthesis and metabolism of ribosomal RNA in randomly dividing cultures have been described[1–5], it is not known whether these processes occur similarly in all stages of the growth cycle. Several investigators working with individual cells have demonstrated that the rate of RNA synthesis increases progressively throughout interphase[6–8]; however, in order to study the synthesis of the different molecular species of RNA large numbers of synchronized cells are necessary. Using a method of synchronization[6,9] which avoids the ambiguities introduced by employing physical and chemical inhibitors, we have related the synthesis of high molecular weight and ribosomal RNA to various stages of the cell's life-cycle.

Suspension cultures of synchronized *S*3 HeLa were prepared by preferentially detaching mitotic cells from monolayers grown in Eagle's spinner medium[10] supplemented with 3·5 per cent calf and 3·5 per cent foetal calf serum. Rates of synthesis of total RNA, DNA or protein were obtained by labelling 0·5-ml. aliquots of synchronized

cells in suspension for 30 min with either 0·2 μc. of ^{14}C-uridine (30 mc./mmole), 0·5 μc. of ^{14}C-thymidine (30 mc./mmole), or 1 μc. of a mixture of ^{14}C-threonine, valine and phenylalanine (100–300 mc./mmole) (New England Nuclear Corporation). The incorporation of isotope was stopped by chilling, the cells washed free of serum with Earle's saline, and the pellets treated with 5 per cent trichloroacetic acid (TCA). The acid-precipitable material was collected on Millipore filters and analysed for radioactivity in a low background gas flow counter (Nuclear-Chicago). All determinations were carried out in duplicate with a replication error of less than 10 per cent.

To characterize the species of RNA being formed, cells were exposed to ^{14}C-uridine for either 20 min or 2 h, washed in Earle's salts, resuspended in hypotonic buffer (10^{-2} M NaCl, 10^{-2} M *tris*-HCl *p*H 7·4, $1{\cdot}5 \times 10^{-3}$ M $MgCl_2$) and disrupted with 1 per cent sodium dodecyl sulphate (SDS). In some experiments cytoplasm was separated from nuclei by Dounce homogenization following hypotonic shock[11,12]. Unlabelled HeLa cell cytoplasm was added to provide optical density markers and the lysates were analysed by centrifugation at 20° C through 15–30 per cent linear sucrose gradients containing 0·5 per cent SDS, 0·1 M NaCl and 5×10^{-3} *tris*-HCl *p*H 7·4. The ultra-violet absorbancy at 260 mμ was monitored with a recording spectrophotometer (Gilford) and acid-precipitable radioactivity was determined by making samples 12 per cent with TCA and processing them as already described here. Alternatively, cells were washed, resuspended in hypotonic buffer, added to HeLa cell cytoplasm, and the RNA extracted with hot phenol and SDS[1]. While both techniques have given essentially the same results if less than 10^6 cells are used, extraction with SDS alone is more convenient and gives more reproducible data.

The metaphase index was determined by sedimenting a small aliquot of cells and counting the fraction in metaphase.

The life-cycle of mammalian cells has been divided into the interval between mitosis and DNA synthesis (*G*1), the period of DNA synthesis (*s*), and the interval between DNA synthesis and mitosis (*G*2)[13]. In order to relate the rates of synthesis of high molecular weight and ribosomal RNA to these stages, it was important to establish the rate of total RNA synthesis throughout interphase and its relationship to the synthesis of DNA and protein in our strain of HeLa *S*3 (Fig. 1). In general, the progressive increase in the rate of total RNA synthesis throughout interphase as well as the character and duration of the DNA synthesis curve confirm the findings which have been reported for single cells[6–8]. The depression of RNA and protein synthesis in the presence of a high mitotic index immediately after synchronization (55 per cent of the cells in metaphase, 45 per cent in anaphase or telophase) and between 14 and 17 h, is consistent with autoradiographic investigations[14–17] and with our recent observation that the number of polysomes are greatly decreased during mitosis[18].

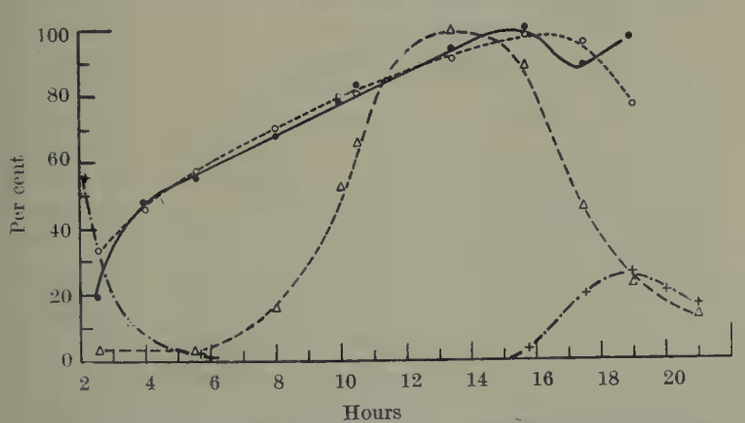

Fig. 1. Synthesis of RNA, DNA and protein. 10^5 cells in 0·5 ml. aliquots were incubated at the indicated times with ^{14}C-uridine, ^{14}C-thymidine or a mixture of ^{14}C-valine, ^{14}C-threonine and ^{14}C-phenylalanine for 30 min and processed as described in the text. The RNA data have not been corrected for the 8–10 per cent of the acid-precipitable ^{14}C-uridine counts which are found in DNA during the period of significant DNA synthesis. ×, Metaphase index; △, ^{14}C-thymidine incorporation; ●, ^{14}C-uridine incorporation; ○, ^{14}C-amino-acid incorporation

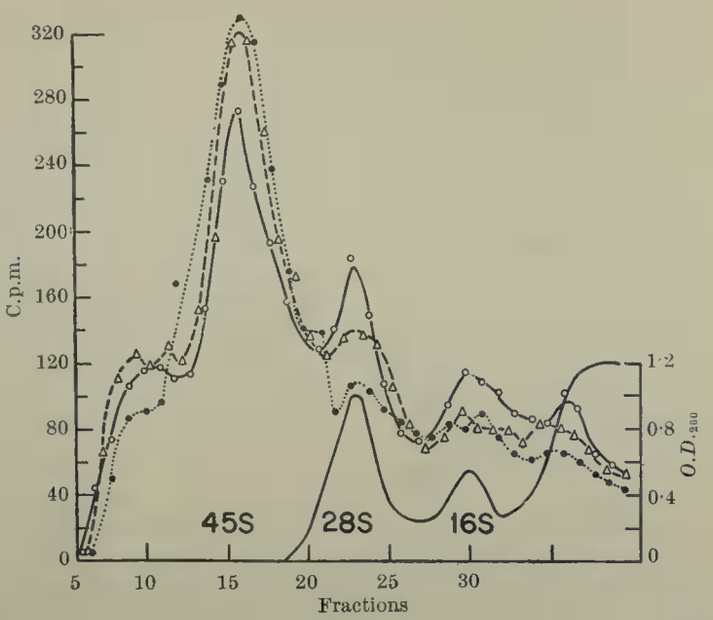

Fig. 2. Synthesis of 45*S* RNA in *G*1, *s* and *G*2. Separate preparations of synchronized HeLa cells were collected at 2, 9 and 15 h prior to labelling so as to allow simultaneous processing of *G*1, *s* and *G*2 populations. Four ml. of cells at 2×10^5 cells/ml. were incubated with 5 μc. ^{14}C-uridine for 20 min. Following washing, the cells were mixed with 1 ml. of unlabelled HeLa cell cytoplasm from 2×10^7 cells. The mixture was extracted with 1 per cent SDS and layered on linear sucrose gradients which were centrifuged for 14 h at 19,000 r.p.m. in the *SW* 25 swinging bucket rotor. ○, *G*1; △, *s*; ●, *G*2

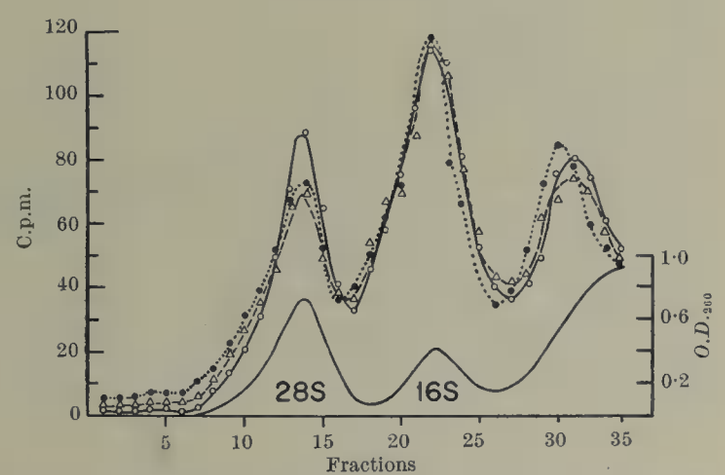

Fig. 3. Synthesis of cytoplasmic RNA. Cells from *G*1, *s* and *G*2 were prepared as described in the legend of Fig. 2. Two ml. from each stage was exposed to 2·5 μc. ^{14}C-uridine for 2 h, washed, and mixed with 2×10^7 unlabelled HeLa cells. Cytoplasm was prepared as described in the text and following extraction with 1 per cent SDS analysed on sucrose gradients centrifuged at 23,000 r.p.m. for 16 h. ○, *G*1; △, *s*; ●, *G*2

Since mitosis lasts 30–45 min, an appreciable mitotic index persisting for several hours indicates the loss of synchrony which characteristically occurs during one division cycle. It is clear that this loss of synchrony makes it impossible to obtain a uniform population of cells which have completed DNA synthesis but have not yet entered mitosis (*G*2). Because of this, cell populations which we will call *G*2 are in fact contaminated with significant numbers of cells which are still synthesizing DNA or in mitosis, as well as a few which have begun a second interphase.

In randomly dividing cultures of mammalian cells most of the RNA synthesized is ribosomal RNA. Its synthesis begins with the appearance in the nucleus of a high molecular weight RNA with a sedimentation value of approximately 45*S*. After 20–30 min exposure to ^{14}C-uridine, small amounts of newly synthesized 28*S* and 16*S* RNA are also detectable and with longer labelling times 28*S* and 16*S* RNA are found in the cytoplasm in association with the 60*S* and 40*S* ribosomal sub-units respectively.

The 16*S* RNA enters the cytoplasm more rapidly than the 28*S* RNA[1–5].

Figs. 2 and 3 illustrate that these events occur at all stages in the cell-cycle. Fig. 2 shows the results of exposing cells to ^{14}C-uridine for 20 min during *G*1, *s* and *G*2. For more convenient comparison of the relative quantities of the different RNA species, the data have been 'normalized' so that there are the same number of total counts per gradient. Thus, while there is less total RNA synthesized per ml. of cells in *G*1 than in *s*, this is not apparent from the curves as drawn. As can be seen, cells in *G*1, *s* and *G*2 synthesized primarily high molecular weight RNA during the first 20 min of exposure to label, although there was also a small amount of newly synthesized RNA in the 28*S* and 16*S* areas. In the experiment illustrated there is relatively less newly synthesized 45*S* RNA and more 28*S* and 16*S* RNA in *G*1 than in *s* and *G*2, but these differences are variable and of unknown significance; the precise height of the peak, however, is of less significance than the fact that 45*S* RNA is made in large quantity throughout interphase. Analysis of the cytoplasm alone (Fig. 3) further emphasizes the constant pattern of RNA synthesis during the growth cycle; after 2 h exposure to ^{14}C-uridine, 28*S* and 16*S* ribosomal RNA as well as 4*S* transfer RNA are present in the same relative amounts at all stages; 16*S* RNA was consistently present in larger amounts due to its more rapid entry into the cytoplasm. Similar results were obtained when other time points in *G*1 and *s* were examined.

Since it has been shown that RNA is not synthesized on replicating DNA[14,19], it is important to consider whether a decrease in either total or ribosomal RNA synthesis should be detectable during *s* in synchronized HeLa cells. During the time of maximum thymidine incorporation (11·5–12 h), only 5 per cent of the total DNA was synthesized. If the replication of the 400 cistrons responsible for synthesis of ribosomal RNA[20] were a random process, it is clear that we would expect to see, at the most, a parallel 5 per cent drop in ribosomal RNA synthesis; this is essentially our experimental error. Even if the replication of these cistrons were not a random process and occurred during a short interval, lack of perfect cell synchrony would make detection of a decrease in RNA synthesis difficult in our experiment; however, analysis of single cells using short exposures to label and autoradiography might resolve this question. It should be noted that while Reiter and Littlefield[21] reported a decrease in nuclear RNA synthesis during *s* in chick fibroblast cells synchronized with FUDR, it does not appear that their results are applicable to untreated HeLa cells.

Perhaps more interesting is the absence of a significant increase in RNA synthesis during *s*. This clearly implies that either the newly synthesized DNA does not act as a template for RNA synthesis or that the pre-existing DNA is progressively repressed.

We thank Dr. A. Zetterberg for allowing us to see his manuscripts prior to publication.

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HETEROGENEOUS BINDING OF ACRIDINE ORANGE BY POLYRIBONUCLEOTIDES

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ACRIDINE orange (AO) binding of polyribonucleotides results in extensive stacking of the dye molecules in linear arrays along the polymer chain[1–3]. This is attributed to a highly co-operative influence of neighbouring bound dye molecules. The dissociation constant for the first dye molecule is larger than that of the second or subsequent adjacent dye molecules. If the discrepancy between the first and subsequent dissociation constants is great enough, a given polymer chain becomes 100 per cent saturated with the dye before a second polymer chain begins to bind the dye.

Evidence for this quantum type of dye binding has been obtained in adsorption isotherm investigations with *Micrococcus lysodeikticus* and *Bacillus cereus*[4]. The fraction of cells stained orange by the dye (10 per cent of dry weight) is proportional to the amount of dye bound by a given suspension of cells, that is, the percentage saturation of the bacterial suspension is equal to the percentage of bacterial cells saturated 100 per cent by the dye.

The molecular weight of a polynucleotide–dye complex 100 per cent saturated with AO is approximately twice that of the free polymer. Density gradient ultracentrifugation should demonstrate an increase in mobility of the polymer saturated with dye and, indeed, separate the dye–polymer complexes from the free polymer molecules[5]. Polyadenylic, polyuridylic and polycytidylic acids were purchased from Miles Chemical Co., Elkart, Indiana. Polyadenylic acid saturated 50 per cent with AO was centrifuged in a sucrose gradient at 63,500*g* for 17·5 h. Fig. 1 shows the distribution pattern of the polymer (260 mμ) and of the dye (465 mμ). The slowest component, which is essentially free of the dye, has the same mobility as that of the control sample of polyadenylic acid. The faster component with a mobility approximately twice that of polyadenylic acid corresponds to the distribution

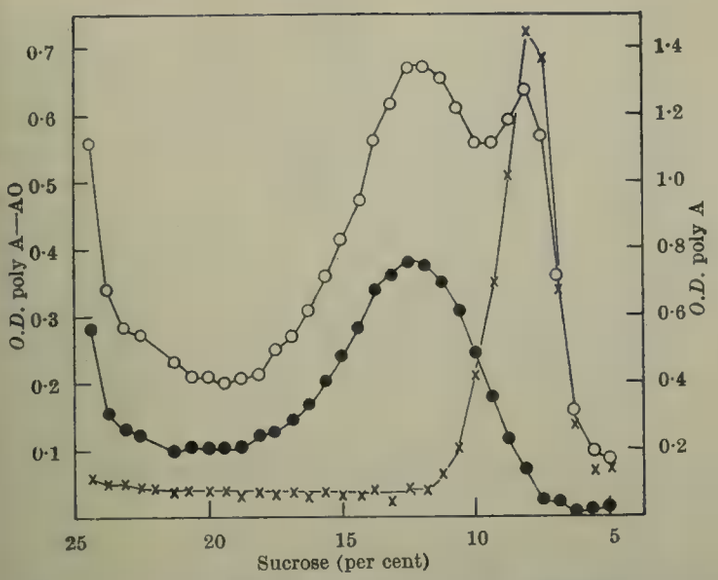

Fig. 1. Density-gradient ultracentrifugation of poly A saturated 50 per cent with acridine orange. One ml. each of 1 mM poly A and 0·5 mM acridine orange in 0·5 mM *tris*, *p*H 7·5, were mixed and layered in a sucrose gradient 5–25 per cent. The preparation was centrifuged at 63,500*g* for 17·5 h. Control was performed under identical conditions without the dye. One-ml. aliquots were moved from the bottom of the centrifuge tube for measurement of their respective optical densities at 260 mμ and 464 mμ. ○, Optical density of poly A–acridine orange mixture at 260 mμ; ●, optical density of poly A–acridine orange mixture at 465 mμ; ×, optical density of poly A control at 260 mμ

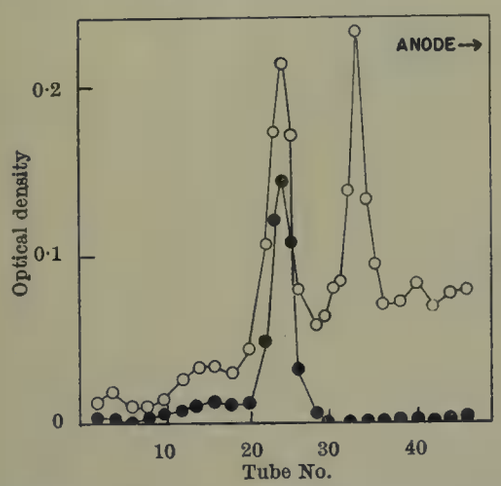

Fig. 2. Density-gradient electrophoresis of poly A saturated 50 per cent with acridine orange. One ml. sample containing 8×10^{-6} M poly A, 4×10^{-5} M acridine orange and 5×10^{-3} N phosphate buffer, *p*H 7·5, in 4 per cent sucrose was layered on a sucrose gradient column 4–25 per cent in 5×10^{-3} M phosphate buffer, *p*H 7·3. Electrophoretic run lasted 3 h at 2 m.amp and 20° C. One-ml. aliquots were removed for measurement of their respective optical densities at 260 mμ and 465 mμ. ○, 260 mμ; ●, 465 mμ

pattern of the dye and, in fact, is 100 per cent saturated with AO. A third component sedimenting to the bottom of the centrifuge tube is an aggregate of the dye-saturated polymers. Similar results have been obtained with polyuridylic and polycytidylic acids. The fraction of polymer molecules saturated with the dye is always proportional to the amount of dye present.

Resolution of copolymers by this method has not been successful, although the peak of dye distribution measured at 465 mμ is always in advance of that of the polymer measured at 260 mμ. The observed mobility of the dye–polymer mixture increases with the degree of saturation of the polymer mixture. Copolymers were synthesized by the action of polynucleotide phosphorylase prepared from *Micrococcus lysodeikticus* cells.

Because AO is a singly charged cation at the *p*H ranges employed in these studies (*p*H 7–8), the fully saturated polymer should have a zero net charge. Therefore, it should be possible to separate the polymer components by electrophoresis. Density gradient electrophoresis studies were performed in a water-jacketed column modified after the apparatus described by Olivera *et al.*[6]. Preliminary studies with zinc-free dye failed to demonstrate any separation of the components. However, with the zinc-dye complex (net charge +3) the polymer can be separated into two components. The faster component demonstrates a bright green fluorescence and the slower component a bright orange fluorescence. The former corresponds to Complex II, the latter to Complex I (ref. 2). Fig. 2 shows the distribution pattern of polyadenylic acid 50 per cent saturated with acridine orange electrophoresed in a sucrose gradient. The slower component is 100 per cent saturated with the dye. The addition of $MgCl_2$ at concentrations comparable with those of the dye reduced or abolished the resolution of the peaks. In contrast, zinc increases the resolution of the peaks.

Fig. 3 gives the distribution pattern of a copolymer of adenylic and uridylic acids with a one-to-one base ratio. Similar results have been obtained with yeast RNA. However, the extent of dye binding by the slower component is considerably less than 100 per cent.

Equal molar mixtures of polyadenylic acid, polyuridylic acid and AO, heated to 100° briefly and cooled slowly when electrophoresed in the sucrose gradient, fractionate into two components (Fig. 4). The faster component contains 80 per cent polyadenylic acid, indicating that the dye has a stronger affinity for polyadenylic acid than for polyuridylic acid.

These results suggest that the capacity of a particular copolymer to bind the dye may be a function of its base composition or sequence. To test this hypothesis, an equi-molar mixture of two copolymers having 24·5 and 86·5 per cent adenylic acid, respectively, was electrophoresed with equi-molar AO in the sucrose gradient. The faster component free of the dye had an adenylic acid content of only 47·5 per cent, indicating that the copolymer containing the higher fraction of adenylic acid preferentially bound the dye.

Similar results have been obtained with methylene blue and acriflavine. Acriflavine appears to provide a better separation of the polymer complexes. Moreover, the fully saturated dye–polymer complex does not migrate from the origin.

These investigations clearly establish for the first time the quantized nature of acridine orange binding by polyribonucleotides containing one to four different bases. Even with RNA there is no evidence of any significant

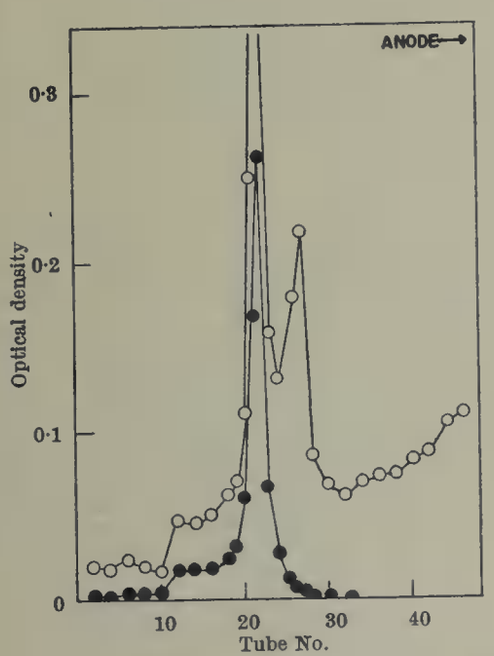

Fig. 3. Density-gradient electrophoresis of poly AU saturated 50 per cent with acridine orange. One ml. sample of 9×10^{-5} poly AU (1 : 1 base ratio), $4{\cdot}5 \times 10^{-5}$ M acridine orange, and 5×10^{-3} M phosphate buffer, *p*H 7·5, in 4 per cent sucrose was applied to electrophoretic column. Electrophoretic run lasted 1 h at 3 m.amp and 4° C. Experimental conditions were the same ▬ in Fig. 2. ○, 260 mμ; ●, 465 mμ

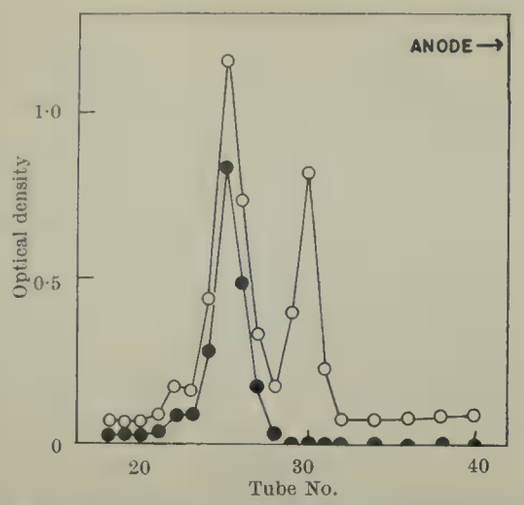

Fig. 4. Density-gradient electrophoresis of equi-molar mixtures of poly A, poly U and acridine orange. One-ml. sample containing 0·36 mM each of poly A, poly U and acridine orange in 4 mM phosphate buffer, *p*H 7·5, was heated briefly to 100° in a water-bath and cooled slowly to 4° before applying to the sucrose gradient. Electrophoretic run lasted 3 h at 3 m. amp and 4° C. Experimental conditions were the same as in Fig. 2. ○, 260 mμ; ●, 465 mμ

random dye binding in which the dimer of the dye predominates[3].

Because of these findings the effects of the dye on the kinetics of polynucleotide phosphorylase must be reinterpreted[7]. In particular the mechanism by which the fully saturated dye–polymer complex stimulates the polymerization reaction apparently involves more than a simple electrostatic effect as postulated elsewhere. The dye titration studies with various polyribonucleotides[2,3] also must be re-interpreted on the basis of a quantized scheme of dye binding.

The second significant observation is the dependence of the dye binding on the base ratios and possible base sequences. This suggests the possibility of using the dye as a reagent for fractionating RNA according to the base composition of the polynucleotides.

The persistent negative electrostatic charge of fully saturated AO–polymer complexes is an unexpected finding which may be accounted for by the presence of a zeta potential of the dye–polymer aggregate. These aggregates are formed reversibly but can be readily demonstrated by density gradient ultracentrifugation. Visual inspection of the electrophoretic column suggests the presence of aggregates. If this is the case the dye–polymer complexes are not in solution but exist as the solid component of a two-phase system. This would provide a physical basis for the existence of a zeta potential on the surface of these aggregates. Attempts to reverse the charge of the complexes by the addition of equi-molar Zn^{2+} were unsuccessful. During the electrophoretic run the Zn^{2+} contained in the dye migrated to the cathode, whereas the dye–polymer complex migrated to the anode.

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NUCLEOTIDE PEPTIDES IN YEAST

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THE occurrence of complexes containing nucleotides and amino-acids has recently been reviewed[1]. The best known of these are the amino-acid derivatives of transfer-RNA and the corresponding peptide derivatives now regarded as ribosomal-bound intermediates in protein synthesis[2,3]. Complex mixtures of less-well-characterized compounds of low molecular weight are present in aqueous-ethanolic and trichloroacetic acid extracts of yeasts[4–11] and similar mixtures have been reported from other micro-organisms, green plants and various mammalian tissues[1,12–14].

Davies and Harris[9,10] isolated several compounds from yeast and assigned structures involving phosphoanhydride bonds between the 5′-phosphate of uridylic acid and the carboxyl group of alanine. The extracts obtained from yeast were clearly very complex[10,11], but many of the components had broadly similar amino-acid and nucleotide composition and reduced periodate rapidly. They were thus thought to possess 5′-phosphoanhydride bonds. After mild treatment with alkali the ability of these mixtures to reduce periodate increased. This was interpreted as indicative of the presence of nucleotide peptides possessing ester bonds between the 2′- or 3′-hydroxyl group of ribose and the carboxyl group of an amino-acid[15]. Similar structures had been suggested by Koningsberger *et al.*[4,6].

During re-investigation of these preparations, attempts were made to correlate nucleotide (calculated as uridylic acid from the extinction at 260 mμ), ribose (determined by the method of Mejbaum[16] after bromination of the sample[17]), amount of periodate reduced[15] and number of alkali-labile 'activated' bonds. The latter were estimated by reaction with hydroxylamine at neutral *p*H before and after mild alkaline hydrolysis followed by determination of the hydroxamate formed[18]. The values thus obtained did not exceed 3 μmole/g yeast in any of the samples tested. The nucleotide- and ribose-levels were always in reasonable agreement and in excess of the alkali-labile 'activated' bonds. The initial uptake of periodate was, however, erratic and invariably in excess of the total ribose content. In some samples even the increment in consumption of periodate resulting from mild alkaline hydrolysis was greater than the total ribose in the sample. As purification proceeded, the correlation improved, but only after numerous procedures were the results observed from some fractions consistent with ribose being the only moiety oxidized by periodate. These results indicated the desirability of characterizing further components of the mixture, in particular a 2′- or 3′-ribose ester. One such class of compounds might be expected with a free 5′-phosphate group. These would be relatively acidic and were therefore sought in that small proportion of nucleotide–peptide concentrate which was retained by 'Dowex-1' (formate) in the presence of 0·1 M sodium acetate buffer, *p*H 5 (ref. 10), a fraction hitherto not investigated in detail.

Elution of the 'Dowex-1' (formate) column with 0·1 N formic acid and subsequent rechromatography of poorly resolved regions on the same resin gave rise to thirteen distinct fractions. Each of these separated into two to four further fractions when passed through 'Sephadex *G*-25'. Certain of the latter, which absorbed ultra-violet light, reacted with ninhydrin and exhibited an increased ability to reduce periodate after mild treatment with alkali, were

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selected for detailed examination. One such investigation is described in some detail as illustrative of the results obtained.

The sample had λ_{max} 255 mμ, λ_{min} 235 mμ and appeared as a single ninhydrin-positive spot with coincident absorption of ultra-violet light on thin-layer chromatography on cellulose in three solvents (0·1 N hydrochloric acid, solvent *a*; propan-2-ol–water, 7 : 3 v/v, solvent *b*; saturated ammonium sulphate–0·1 M sodium acetate *p*H 7·5–propan-2-ol, 40 : 9 : 1 v/v). It failed to move or separate on paper electrophoresis (0·1 M sodium acetate buffer, *p*H 4, 36 V/cm). After treatment at *p*H 10 for 16 h at room temperature it was possible to separate the ninhydrin positive and ultra-violet absorbing material by paper chromatography (solvent *b*).

The peptide moiety was eluted and purified by paper electrophoresis and chromatography before being hydrolysed (6 N hydrochloric acid, 100°, 16 h, sealed tube). Amino-acids, determined by the method of Spackman, Stein and Moore[19], were present in the hydrolysate in the approximate ratio glutamic acid : glycine : serine : alanine, 3 : 2 : 1 : 1 (Table 1) together with traces of aspartic acid, threonine and lysine. Reaction of the original material with 'dansyl' chloride[20] and subsequent hydrolysis yielded a single derivative tentatively identified as dansyl-alanine by chromatography[21].

Table 1. Analytical Data on a Selected Nucleotide Peptide Fraction

		Moles/mole ribose
Nucleotide (calculated as G)		1·2
Periodate consumed:	(*a*) Initially	0·9
	(*b*) After hydrolysis at *p*H 10	1·03
	That is, ribose-ester bonds	0·13
Amino-acids:	Glu	0·063
	Gly	0·05
	Ser	0·026
	Ala	0·022
	That is, total peptide	*c.* 0·024

The nucleotide moiety was also subjected to hydrolysis (0·3 N potassium hydroxide, 37°, 16 h) after elution from the paper. Paper chromatography of the products (*n*-butanol–acetic acid–water; 4 : 1 : 1 v/v) revealed two components (*A* and *B*, R_F = 0·2 and 0·38 respectively) with distinctive ultra-violet absorption spectra (Table 2). Neither component could be identified when compared with all the available authentic bases or nucleoside monophosphates either by paper or thin-layer chromatography or by electrophoresis. Component *A* was then subjected to acid hydrolysis (*p*H 2, 100°, 10 min) and the products chromatographed on paper (*n*-propanol–ethyl acetate–water, 7 : 1 : 2 v/v) adjacent to unhydrolysed component *A*. On developing with silver nitrate it was apparent that a hexose had been released by acid hydrolysis. This was identified as galactose, together with traces of glucose and mannose, by paper chromatography (pyridine–ethyl acetate–water, 4 : 10 : 3 v/v, and methyl ethyl ketone–acetic acid–water, 9 : 1 : 1 v/v saturated with boric acid) and electrophoresis (0·1 M sodium borate buffer, *p*H 10, 22 V/cm). Finally, the nucleotide remaining after acid hydrolysis was tentatively identified as guanosine 5′-phosphate by eluting from chromatograms of the hydrolysate and re-running on paper (propan-2-ol–6 N hydrochloric acid, 17 : 8 v/v; solvent *c*). Similar treatment of component *B* failed to reveal any acid-labile carbohydrate. The spectral characteristics and chromatographic behaviour (Table 2) of this component therefore suggested that it was a methylated guanosine[22].

Table 2. Spectral and Chromatographic Characteristics of Nucleotides Derived from an Individual Yeast Nucleotide Peptide Fraction

Component	*A*			*B*		
*p*H	2	7	11	2	7	11
Max	252	245	268	256	257	257
	271–4 (infl.)	265–70 (infl.)				
$E_{250m\mu}/E_{260m\mu}$	1·2	1·1	0·95	0·95	0·99	0·99
$E_{280m\mu}/E_{260m\mu}$	0·63	0·79	0·83	0·23	0·74	0·66
*E*Guanosine						
Cellulose thin layer, solvent *a*		2·34			0·85	
solvent *b*		0·79			1·38	
No. 1 paper, solvent *c*		1·4			0·8	

From the data enumerated so far, it is tempting to suggest a structure for this nucleotide peptide such as:

Galactose–*p*G*p*MeG–peptide

Indeed, a rather similar glucose–cytidylic acid–peptide having a qualitatively identical amino-acid composition has recently been isolated from chlamydospores of oat smut[23]. The quantitative data collected in Table 1 are, however, not in agreement with such a conclusion. One might reasonably expect to recover at least 20–30 per cent of the peptide after eluting and re-running several times on paper. In this event there is reasonable agreement between the amounts of peptide and material which reduces periodate released by cleavage at *p*H 10. There is, however, far too little of either ribose-ester bonds or peptide when compared with the total nucleotide content. Furthermore, phosphate analyses were not in accord with this view. Perhaps the best interpretation is that the fraction is a nucleotide derivative of galactose some 5–10 per cent of which bears a peptide attached by a ribose-ester bond.

It is clear that neither the techniques of separation nor the criteria of homogeneity so far used have been adequate. Thus the possibility that the fraction may be considerably more complex cannot be entirely excluded in view of the known strong association of guanine derivatives[24] and the recently reported occurrence of xanthosine monophosphate galactose in close association with nucleotide peptides from *Escherichia coli*[12].

Several other apparently homogeneous ester-linked components were also examined in detail, but in no case were satisfactory quantitative data obtained to support the qualitative findings. In addition, several other fractions from the 'Sephadex' columns which were clearly heterogeneous were fractionated further on DEAE-cellulose according to Tomlinson and Tener[25]. Invariably, in addition to fractions exhibiting typical nucleotide spectra, several other components were obtained with λ_{max} 225–230 mμ; 321 mμ, λ_{min} 267 (*p*H 2 and 7), and λ_{max} 232–233 mμ; 308–310 mμ, λ_{min} 264 (*p*H 9). These latter were not investigated closely, but it was established that they reduced periodate very rapidly. Compounds with these spectral characteristics were also found in considerable amounts in the whole nucleotide–peptide concentrates.

The presence of bound hexoses and materials which react readily with periodate, other than the nucleotide peptides themselves, invalidates the measurement[15] of the proportions of anhydride and ester-linked compounds in these nucleotide peptide concentrates. Thus, the reported changes in these compounds accompanying physiological changes in the cells[14,26–28] cannot be regarded as having any quantitative significance or affording any indication as to the metabolic significance of nucleotide peptides.

One class of nucleotide peptides having ribose-ester bonds might arise by the action of ribonuclease on ribosomal-bound transfer-RNA peptides[3,29] during the extraction procedure or, in cell-free systems at least, by degradation of the transfer-RNA–amino-acid prior to peptide bond formation, a process which inhibits protein synthesis and presumably releases nucleotide peptides[30]. The nucleotide composition of such components should reflect the characteristic terminal sequence of transfer-RNA. Such products would not be intermediates in protein synthesis but might provide an explanation of the apparent connexion between nucleotide peptides and protein synthesis[31–34]. Although investigations of the

incorporation of radioactive amino-acids into protein by systems derived from yeast and dependent on either ribosomes[35] or a heavier particulate fraction[32] have indicated that this incorporation is accompanied by passage of radioactivity into materials which possess the characteristics of nucleotide peptides[32,34], kinetic evidence has failed to demonstrate an intermediary role for these substances in protein synthesis in either system[34]. From the extract of the heavier particulate fraction the nucleotides obtained by alkaline degradation may well have been characteristic of terminal fragments of transfer-RNA[32]. However, it has not proved possible to isolate, from either system, sufficient radioactive material in an adequate state of purity to elucidate the nature of the bond between nucleotide and peptide[34]. No nucleotide peptides having structures compatible with terminal fragments of transfer-RNA have, as yet, been adequately characterized and, to judge from the overall nucleotide composition of the preparations[1,11], it seems likely that only a relatively small fraction could be so derived. Perhaps the most reasonable explanation of an apparent intermediary role in protein synthesis in living cells[33], which is unconfirmed in cell-free systems, is that certain nucleotide peptides are involved in the elaboration of structures which require the integrity of the cell, such as membrane, mitochondria, or cell walls[28].

Although fragments of transfer-RNA amino-acid derivatives inhibit protein synthesis[30], it is not obvious that the analogous peptide derivative could have the similar but apparently specific action required to explain the repression of the induced synthesis of α-glucosidase observed by Koningsberger *et al.*[36]. We have also examined the latter phenomenon using a strain of *Saccharomyces cerevisiae* (National Collection of Yeast Cultures No. 366). Cells in early log phase and protoplasts derived therefrom showed an induction pattern almost identical with that of stationary phase cells of this strain[37]. Initially, in the presence of rather crude preparations containing nucleotide peptides our observations were very similar to those of Koningsberger *et al.*[36]. Attempts to fractionate these preparations by the techniques already outlined here, however, afforded rather confusing results, several fractions exhibiting similar activity being obtained. Repeated passage through 'Sephadex *G*-25' and final removal of traces of salts on ion retardation resin ('Bio-Rad. *AG*11*A*8') led to a gradual loss in activity, suggesting either that the active material is rather labile or that it is not, in fact, nucleotide peptide. One of the most surprising observations was that one fraction which had been kept cold throughout the preparative manipulations and exhibited only very slight activity was able to inhibit completely the synthesis of maltase in protoplasts after it had been warmed at 60° for 10 min.

We also have independent evidence that may indicate the presence of substances having repressor-like activity in the readily extractable pool of low-molecular-weight substances from yeast. The sequential induction of the components of the maltose utilizing system in a further strain of *Saccharomyces cerevisiae* (N.C.Y.C. 240 M), in which maltase activity only became apparent some 3·5 h after the addition of the inducer, was described earlier[37]. It has since been found that this behaviour is dependent on the temperature at which the cells are washed prior to addition of the inducer. The delayed induction, in fact, occurs only after the cells have been washed at 0°–5°, whereas washing at 20–25° results in induction within less than 2 h. The latter effect can, however, be largely offset by an increase in the concentration of glucose in the inducing medium. One possible explanation is that washing at the higher temperature removes substances with repressor-like activity. Freeze-dried washings are, however, without effect on the induction in whole cells or protoplasts when observed manometrically. Nevertheless, it is possible that the repressor is degraded on excretion by enzymes located in the cell wall[38]. Indeed, this could also explain the observation[36] that repressor activity can be demonstrated on protoplasts but not on whole cells. These observations may, perhaps, be connected with the phenomenon of shock excretion of amino-acids and nucleotides[39] which is enhanced by elevated temperatures. Conceivably, some of the excretion products may be derived from nucleotide peptides; indeed, it is not impossible that one function of shock excretion is removal of repressor substances prior to an adjustment of enzyme levels in response to a change of environment. Against these ideas, however, radioactivity from nucleotide peptides obtained from yeast grown in the presence of radioactive amino-acids failed to enter either cells or protoplasts to any significant degree[34], and a synthetic nucleotide peptide[40] linked by a 5′-phosphoanhydride bond failed to support yeast growth despite the fact that the tetrapeptide moiety alone was able to serve as sole nitrogen source[41].

The precise significance of these results remains uncertain. There seems little doubt that low-molecular-weight substances having a repressor-like activity exist in yeast, but whether this observation is distinct from that of catabolic repression and is more specifically associated with nucleotide peptides seems rather doubtful. The variety of structures which have been observed make it unlikely that one can assign a single function to nucleotide peptides. Some may fulfil a genuine intermediary role in the assembly of cellular structures. The picture is a complex one and, for further systematic study, requires the application of additional separation techniques and the development of more rigid criteria of homogeneity.

We thank Dr. A. H. Cook for his advice.

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A MOLECULAR MODEL FOR A SODIUM PUMP

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ALTHOUGH there are considerable experimental data relating to the exclusion of Na^+ and accumulation of K^+ in living cells, our knowledge of the mechanism is rudimentary and the molecular basis for this phenomenon remains unknown. Excellent reviews[1,2] stressing these deficiencies continue to be published. From the work of Skou, Post, Glynn and Whittam during 1957–61 there is strong evidence that the enzyme system known as 'Na^+ and K^+ dependent adenosine triphosphatase' is closely associated with the active transport of sodium and potassium ions across cellular membranes.

In summary, the salient characteristics which relate this enzyme system to the movement of Na^+ and K^+ are:

(1) The enzyme system is membrane bound, and virtually ubiquitous in animal cells[3,5,7].

(2) It requires the presence of both Na^+ and K^+ ions for activation—a well-known requirement for ion transport in whole cell or tissue systems *in vitro*[6–8].

(3) In the erythrocyte, enzyme activation occurs only when Na^+ is on the 'inside' and K^+ is on the 'outside' of the cell[9,10].

(4) Na^+ and K^+ activated ATPase is specifically inhibited by the cardiac glycoside ouabain, known to be a potent inhibitor of cation transport both *in vivo* and *in vitro*[6,7,11].

(5) Kinetic examination of the reaction *in vitro* shows activation by substrate and ions, and inhibition by ouabain at concentrations compatible with 'physiological conditions'[7,12].

Studies by Charnock and Post[13,14], Rose[15] and others[1,16] have suggested the following reaction mechanism for the hydrolysis of ATP by this enzyme in the presence of Mg^{++}, Na^+ and K^+:

$$\text{ATP} + \text{Enzyme} \overset{Na^+}{\rightleftharpoons} \text{E} \sim \text{P} + \text{ADP} \qquad (1)$$

$$\text{E} \sim \text{P} + \text{H}_2\text{O} \xrightarrow[\text{Ouabain}]{K^+} \text{E} + \text{P}_i \qquad (2)$$

The initial reaction step consists of the rapid reversible formation of a phosphorylated complex. This step is stimulated by Na^+ ions, and no other ion appears able to replace Na^+. The formation of this complex is followed by an almost irreversible decomposition to P_i and free enzyme. This latter reaction is stimulated by K^+ but not Na^+ ions; K^+ can be replaced with either NH_4^+, Li^+, Rb^+ or Cs^+. It is this decomposition which is inhibited by the cardiac glycoside ouabain. The chemical nature of the phosphorylated complex is not yet known, although it seems certain that it is phosphoprotein and not phospholipid[13–17]. Kinetic data from this laboratory also support this general reaction mechanism. The K_m for ATP is 2·8 mM; ADP acts as an inhibitor. Little consistent inhibition is apparent with concentrations of phosphate as high as 30 mM (ref. 12).

Although the details of the reaction mechanism of this membrane-bound ATPase have been extensively studied, the means by which this enzyme effects a vectorial transport of Na^+ and K^+ is unknown. Further advances in understanding the mechanism of Na^+ and K^+ transport therefore require information about how such an ATPase might transport Na^+ against the concentration gradient from the inside of the cell, coupled with the movement of K^+ in the opposite direction. The purpose of this article is to present a molecular model for the directional transport of these ions based on what is known of the ATPase reaction mechanism, and which is consistent with knowledge about Na^+ and K^+ transport characteristics in the whole cell or tissue.

It has been generally accepted that the ATPase enzyme itself is membrane bound. We have recently obtained evidence that the enzyme preparation obtained from guinea-pig kidney cortex consists entirely of bilaminar membrane structures, many of which are vesicular, and which are thought to be derived from the endoplasmic reticulum of the kidney cells[18].

We propose, therefore, to develop the model on the basis of these bilaminar membrane fragments and take as our starting point the configuration of the unit membrane proposed by Danielli. This unit contains a double protein layer, with lipid interposed. We will consider the inner facing protein structure to contain the ATPase enzyme. This belief is supported by the observations of Whittam that hydrolysis of ATP only occurs in the red cell ghost when the ATP is intracellular[10].

If, in the intact cell, these enzyme membrane fragments were associated with cell surface or its prolongations into the cell and the endoplasmic reticulum[19–21], then it is reasonable to suppose that the outer protein surface is directed toward the extracellular compartment.

The inner protein structure containing the ATPase must now be visualized as a highly ordered, polarizable chain, with many surface orientated cationic and anionic groups. Among the groups are situated a much smaller number of amino-acid groups that constitute the active centre of the ATPase enzyme itself (Fig. 1). The justification for this type of approach to biologically active protein structure has been developed in some detail by Ling[22], who refers to the active centre as the cardinal site.

In the first state of the protein enzyme which we will consider, the model presupposes that the fixed anionic sites will show no strong preference for either Na^+ ions or K^+ ions, but will form associated ion pairs with either ion. The proportion of ion pairs formed by Na^+ will depend on the Na^+ concentration relative to the K^+ concentration. To initiate operation of the 'sodium pump' a critical number of the protein anionic sites must be occupied by sodium ion pairs. Thus we propose to start with the concept that the operation of the 'pumping cycle' has an inherent feed-back control. The enzyme cycle is stimulated by raising Na^+ concentration and depressed as this concentration falls and the K^+ concentration rises. As a result of sufficient sodium ion pair formation, there is a redistribution of electron density throughout the macro-molecule, and this occurs as a result of both direct and indirect interaction between Na^+ and the protein.

As in the protein fixed-charge model developed by Ling[22], it is considered that the short-range interactions predominate in importance, and contribute in particular to the difference between the sodium and potassium

protein ion pairs. The inductive effect of the cation on the fixed-charge system will be additive and hence the effect of the sodium ion will bear a relation to the number of fixed anionic sites occupied by sodium ions.

Because the protein will also contain surface orientated cationic groups it will also form associated ion pairs with anions at these sites. The interaction between anion and the protein will also effect the induction of charge by cation–anionic site ion pairs. Thus when sodium and potassium are present as fluoride salts *in vitro* the ATPase activity is virtually abolished[12]. In this model we imply that the highly electro-negative fluoride anion is capable of preventing the inductive changes produced by Na^+ adsorption and which are necessary to initiate operation of the enzyme.

The redistribution of electron density induced by sodium ion pair formation is focused in the neighbourhood of the cardinal sites, and, as a result, this centre now reacts more actively with the substrate (ATP) to form the phosphorylated intermediate (Fig. 1). This step corresponds to the biochemical reaction sequence:

$$\begin{matrix} \text{Na} & & \text{Na} & \\ \cdot\cdot & + \text{ATP} \rightleftharpoons & \cdot\cdot & + \text{ADP} \\ \text{E} & & \text{E}\sim\text{P} & \end{matrix}$$

As a result of the phosphorylation of the cardinal site, there is a further inductive effect, and a redistribution of electron density along the polarizable chain of the protein. This produces a folding or deformation of the protein as well as a change in the adsorption energy of the fixed anionic site.

The deformation of the enzyme results, in the model, in a rotation outwards of many of the fixed anionic sites, but leaves the phosphorylated site 'anchored' and still inward-facing (Fig. 2). There are many ways in which this deformation might produce such a change in orientation of the molecule. We consider there is evidence for believing that the whole membrane structure is concerned in the transport process, although not directly part of the enzyme.

It is interesting to note that a recent paper by Tanaka and Abood has suggested that lecithin is required to restore activity to this enzyme after treatment with deoxycholate[23]. We would interpret this finding as suggesting that the lipid is a necessary part of the enzyme system, although not involved in the chemical reaction itself.

The deformation suggested, therefore, implies unfolding or lengthening the inner enzyme protein chain with subsequent rotation of the membrane fragment about the central lipoid core. For a membrane unit of 1000 Å long and 60 Å wide, and assuming that the axis of rotation is in the centre of the lipoid core, a change in length of about 50 Å would be required to produce a single 2π spiral.

Because of the adsorption energy changes associated with this phosphorylation and subsequent enzyme deformation, a marked preference for K^+ is now exhibited by the cation binding groups, and the Na^+ at these sites is exchanged for K^+ (Fig. 2). The previous rotation of the membrane now implies that this exchange occurs extracellularly, hence Na^+ has been transported from the intracellular to the extracellular environment. The adsorption of K^+ on to these sites while the macromolecule is in this state of 'electron strain' again redistributes the electron density. In the region of the

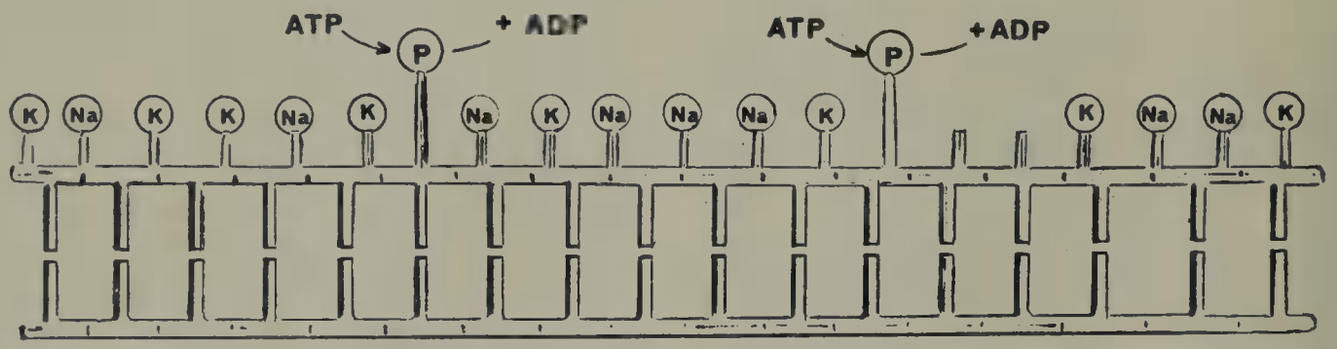


Fig. 1. Diagrammatic representation of a unit segment of membrane in which the horizontal sections depict the inner and outer protein layers, and the vertical broken units between the protein represent the central phospholipid complex described by Danielli. Projecting from the inner coil are shown the surface orientated anionic groups of the protein with which Na^+ and K^+ have formed associated ion pairs. The longer vertical projections represent the amino-acid groups which constitute the active centre of the enzyme. In this figure sufficient sodium ion pairs are considered to have formed to allow the active centre to react with ATP, and form the phosphorylated intermediate. Although the figure shows ATP reacting directly at the cardinal site it has long been established that magnesium ions are required for this reaction. Mg^{2+} has been omitted from the figure for clarity

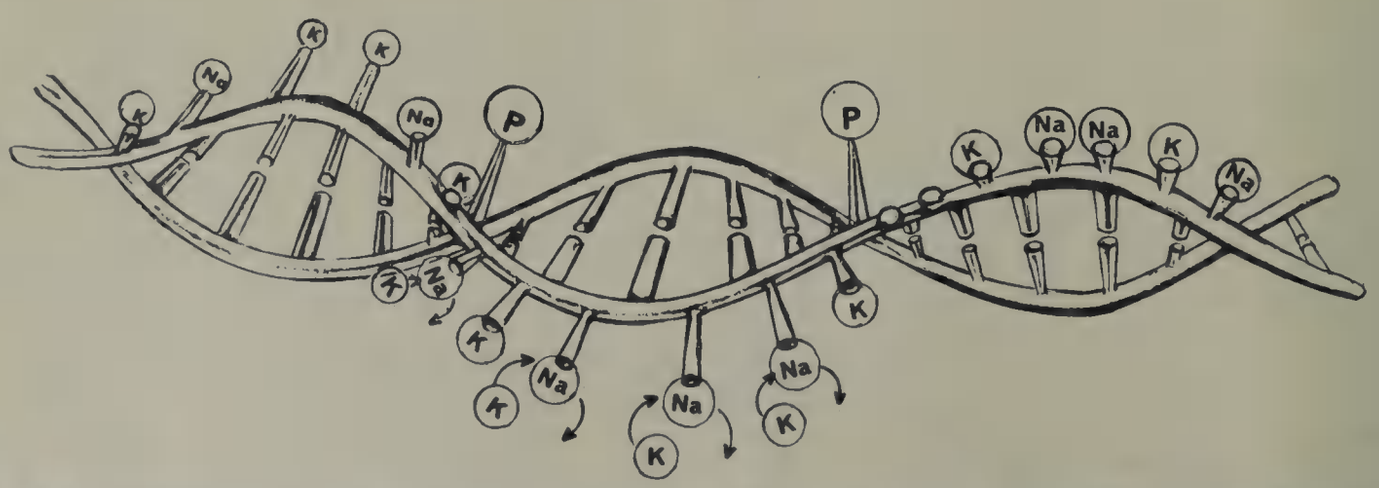


Fig. 2. Representation of the postulated effects of phosphorylation of the active centre of the enzyme. Elongation of the inner protein surface has produced rotation of these protein molecules about the centre of the lipoid core. The site of the phosphorylated active centre is shown as resisting this rotation. The surface orientated anionic groups with their associated ions have rotated outwards following the elongation and twisting of the protein molecule. K^+ for Na^+ exchange is now shown to be taking place at sites of Na^+ adsorption to these groups

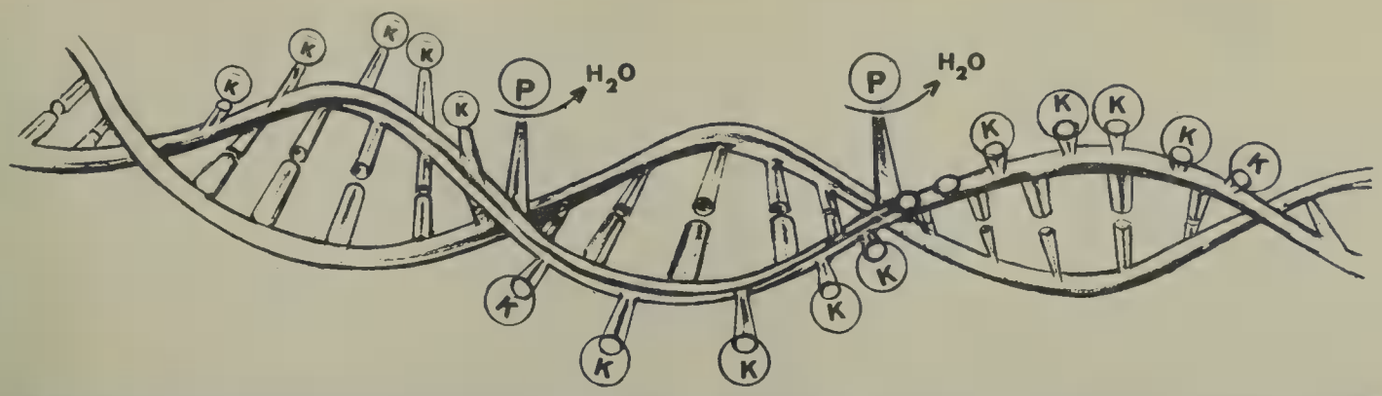


Fig. 3. Diagram showing that, following the K^+ for Na^+ exchange illustrated in Fig. 2, the inductive changes of electron density which take place in the elongated protein molecule lead to changes of field strength in the region of the phosphorylated active centre. As a result of these changes the phosphorylated centre is now readily attacked by water with cleavage of phosphate from the protein

cardinal sites this alteration in electron distribution renders the phosphate groups more vulnerable to attack by water and cleavage of the phosphate bond occurs with the liberation of orthophosphate.

The liberation of phosphate from the phosphoprotein allows the molecule to return to its initial shape, and as a result the anionic binding sites with their adsorbed K^+ rotate inwards toward the cell centre. Within the cell some of these adsorbed K^+ ions are now displaced by Na^+ and the cycle of reaction begins again.

These last hypothetical steps correspond with the second step of enzyme reaction sequence:

$$E \sim P + H_2O \xrightarrow{K^+} E + P_i \qquad (2)$$

Thus the cycle results in net transfer of Na^+ from inside the cell to the outside, coupled with the transport of K^+ in the opposite direction, during which process ATP is hydrolysed to ADP and orthophosphate.

Because there is evidence that the site of ouabain inhibition is at reaction (2) in the sequence given above[13], it seems unlikely that ouabain acts directly at the active centre of the ATPase enzyme. Ouabain does not prevent the formation of the phosphoprotein intermediate but appears to prevent its decomposition[13,14].

For the toxicity and action of cardiac glycosides on ion transport certain features of cardiac glycoside molecule appear essential[24,25]. These features include a *cis*-configuration of the *C–D* ring function, the presence of a β-hydroxy group at C_{14} and an unsaturated lactone ring at C_{17}. A conformational representation of ouabain is shown in Fig. 4. Although the orientation of the molecule

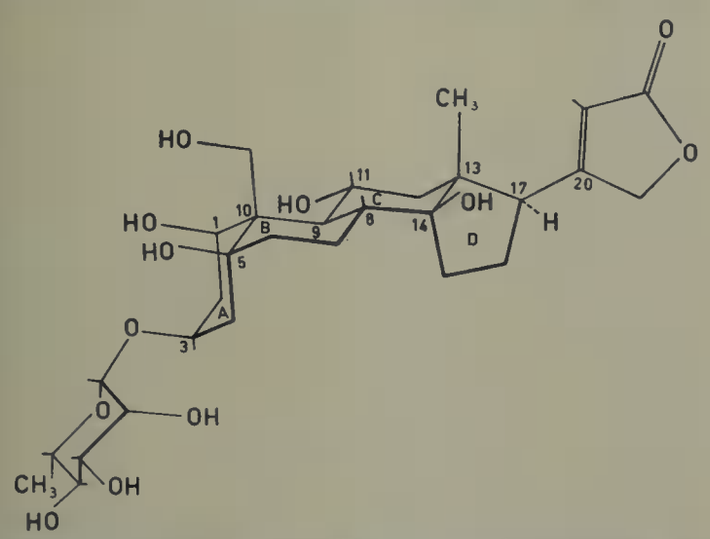


Fig. 4. Conformational representation of ouabain (3-O-β-L-rhamnopyranosyl-1β,3β,5,11α,14,19-hexahydroxy-5β-card 20 (22)-enolide). We are indebted to Dr. J. Mills, C.S.I.R.O. Division of Biochemistry and General Nutrition, Adelaide, for this interpretation of the structure. Rotation of the rhamnose molecule will produce a configuration in which the undersurface of the molecule is hydrophobic, and the upper surface contains projecting OH and =O groups. We envisage that these groups form hydrogen bonds with the extended surface of the inner membrane protein containing the ATPase

will depend on the positions adopted by the lactone ring at C_{17} and the sugar moiety at C_3, as both groups are free to rotate, a conformation could exist where the ketone group and the β-hydroxy at C_{14} face away from the same surface of the ouabain molecule. When oriented in this position, these groups might form by hydrogen-bonding the primary attachment of the ouabain molecule to the surface of the ATPase enzyme.

In addition, a cluster of OH groups exist both on the *AB* rings of the aglycone and on the sugar moiety of ouabain. These groups could also form hydrogen-bonds with the protein enzyme surface. Attachment of ouabain to the protein enzyme by the separated clusters of OH-groups would act, in the model presented in this paper, by maintaining the deformed state of the membrane shown in Figs. 2 and 3. This interaction would prevent both release of phosphate from the active centre, and operation of the deformation–restoration cycle required to move Na^+ and K^+ ions in the directions required.

In the absence of direct experimental evidence, such an explanation for the mechanism of ouabain inhibition must remain entirely speculative.

The model presented in this article is an attempt to combine data about the '$Na^+ + K^+$ activated ATPase' into a consistent model which seeks to explain the means by which such an enzyme could produce vectorial Na^+ and K^+ transport.

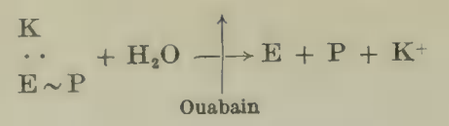
A useful model should be fragile, and ought to suggest the appropriate tests as to its own validity. We believe this model suggests at least two general questions about '$Na^+ + K^+$ activated ATPase' with respect to its capacity to transport these ions.

First, Do transition states of the enzyme exist with selective Na^+ or K^+ binding capacity ? The model, in fact, suggests that selective K^+ binding occurs when the enzyme is in the phosphorylated state (Fig. 3). Recently an answer to this question has been sought using a histochemical electron microscope technique. With this we have been able to show what is believed to be evidence of markedly increased Cs^+ binding by the enzyme membrane during the existence of the phosphoprotein transition state[18]. Because Cs^+ can replace K^+ completely in the enzyme reaction[2,6] we believe that this implies that K^+ binding will occur under the experimental conditions that demonstrated Cs^+ binding. As Cs^+ has a greater electron density than K^+ it is consequently easier to visualize using the electron microscope.

This evidence has necessitated the inclusion of a further step in the reaction sequence to account for a potassium for sodium exchange.

$$\begin{matrix} Na \\ \cdot\cdot \\ E \end{matrix} + ATP \rightleftharpoons \begin{matrix} Na \\ \cdot\cdot \\ E \sim P \end{matrix} + H_2O$$

$$\begin{matrix} Na \\ \cdot\cdot \\ E \sim P \end{matrix} + K^+ \rightleftharpoons \begin{matrix} K \\ \cdot\cdot \\ E \sim P \end{matrix} + Na^+$$



A second question which the model poses is: Does a measurable or detectable degree of molecular deformation occur during or following the formation of the phosphoprotein? One approach to the solution of this question requires direct biophysical examination by such techniques as X-ray diffraction, flow birefringence or light scattering. We have recently been able to show that the particulate enzyme gives a clear orientated X-ray diffraction pattern although as yet we have obtained insufficient resolution to determine the presence of significant deformation[26].

A more indirect approach would be to attempt an estimate of entropy change associated with the hydrolysis of ATP by this enzyme. The model predicts that the entropy values should be high for the ouabain-sensitive Na^+ and K^+ ATPase, and much less for the ouabain-insensitive ATPase which is always associated with our preparations.

Other workers interested in '$Na^+ + K^+$ activated ATPase' have speculated about the mechanism of ion transport by this enzyme. Some authors have considered that either ATP itself, or the phosphate group of the labile phosphoprotein, acts directly as the ion carrier[27]. However, most investigators have treated the enzyme as a polyanionic macromolecule the ion selectivity of which is determined by the turnover of labile phosphate groups[1,2]. Eisenmann[28] has evolved characteristics for such a selective ion binding system which is based on the field strength of the anionic site, and Ling[22] has developed this type of approach with considerable rigour as the basis of specialized protein function in general.

Working with the '$Na^+ + K^+$ activated ATPase' from the electric organ of the electrophoresis, Albers, Fahn and Koval[29] have suggested a model with spatial separation of three enzyme steps. Their model, however, suggests that Na^+ and K^+ movement is coupled with the translocation of phosphate from inside the cell, but fails to account, however, for the return of the phosphate to the cell.

All these models have sought to explain the changing selectivity of binding sites associated with the enzyme 'ion carriers', but none of them has attempted to include an explanation of the vectorial nature of the ion movement, or the highly selective inhibition of both the enzyme and ion transport by the cardiac glycosides. Although the model presented in this article will undoubtedly be proved to be inconsistent as new facts emerge, we believe that it will help to suggest the experiments necessary to uncover these new facts.

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Relictogobius kryzhanovskii AND THE PENETRATION OF MEDITERRANEAN GOBIES INTO THE BLACK SEA

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THE Ponto–Caspian seas of southern Russia are of particular interest to the European ichthyologist in their diverse fauna, with its endemic components, and its relationships to that of the Mediterranean. In addition, nomenclatural problems involving Mediterranean species have resulted from systematic work on this fauna. Fortunately, detailed reviews of both the hydrography and zoogeography of the Black and Caspian Seas have recently become available in English[1–3] together with several comprehensive lists of the fishes of these seas[4–6]. Both seas, and their tributary drainage systems, are populated in varying part by endemic 'Caspian' (= 'Sarmatic' auctt.) faunal elements of more or less stenohaline brackish-water or freshwater animals. The phyletic ancestors of these species evolved from ancient Mediterranean stock with onset of brackish conditions in the vast Sarmatic basin of the South Russian geosyncline after this had become separated in the Upper Miocene from the Mediterranean region of the disappearing Tethys Sea. The establishment of the present connexion between the Black Sea and Mediterranean in the late Pleistocene (post-Würmian[3,7]) led to an increase in salinity of what had previously constituted the semi-fresh Novo-Euxine stage in the history of the Black Sea basin. In consequence, the originally widespread Caspian fauna of the latter now survives as ecological relicts in environments of lower salinity provided by coastal lagoons ('limans'), estuaries of rivers, and the Sea of Azov, and has been largely replaced in coastal and open waters by Mediterranean immigrants which have entered the Black Sea probably within the past 12,000 years[7]. Although there are many Caspian forms restricted in distribution to the Caspian Sea, the existing Caspian fauna of the Black Sea exhibits little endemicity above the subspecific level,

fewer than 5 per cent of the species being absent from the Caspian Sea. This has been attributed to the effect of an earlier Pleistocene (Early Würm/Main Würm interstadial[7], or Riss/Würm interglacial[3]) ingression of Mediterranean waters into the Black Sea basin, when the Caspian fauna of the then hyposaline Ancient Euxine stage of this basin is believed to have been almost completely eliminated from the ensuing marine Karangat Sea[3]. After the closure of this connexion with the onset of the main Würmian glaciation[3,7], salinity of the Black Sea basin became once more reduced and a Caspian fauna, mostly identical in species with the present one, was re-established throughout the sea by immigration from that persisting in the previously isolated basin of the Caspian Khvalynsk stage (via a temporary river, flowing westward, along the Manych depression)[3].

In the modern Black Sea, gobiid fishes form significant elements in both the Caspian and Mediterranean components of the fauna[4–6]. Among those at present included in the former is the monotypic genus *Relictogobius* Ptchelina[8], represented by *R. kryzhanovskii* Ptchelina, known from the type locality, a saline lagoon (20 ‰), loosely walled off from the sea, on the Abrau Peninsula, near Novorossiysk, U.S.S.R. (44° 42′ N., 37° 45′ E.)[8], as well as from Sochi, to the south-east (43° 35′ N., 39° 40′ E.)[10], and from the Gulf of Varna, on the Bulgarian coast (43° 13′ N., 27° 55′ E.)[9]. *Relictogobius* has recently been listed as the only non-freshwater fish genus endemic to the Black Sea[6]. This is in marked contrast to the other 'Caspian' Black Sea gobiids which occur also in the Caspian basin and the populations of which found in the two regions are differentiated into no more than sub-species[3,10]. The Caspian Sea also contains several bizarre genera and species of gobiid fishes lacking in the Black Sea[10]. Had the existence of *Relictogobius* not been apparently overlooked, its level of differentiation suggested by generic status could have provided evidence for persistence through the marine Karangat era of Ancient Euxine forms the survival of which into the Novo-Euxine phase has been the subject of controversy[2,3].

However, while compiling a check-list of European gobies (unpublished), I was impressed by the similarity between published illustrations[8,9] of *R. kryzhanovskii* and specimens of the widely distributed Mediterranean *Gobius quadrivittatus* Steindachner, hitherto unrecorded from the Black Sea. Although the type specimens of the former were destroyed at Novorossiysk during the Second World War[11], the original detailed account leaves no doubt that the two species are identical. Both are small (up to 65·5 mm), elongate gobiids, with about 60–70 rows of cyloid scales in lateral series, a tubular posterior nostril, naked nape and breast, canine teeth, no free pectoral fin-rays, and in coloration show an intricate pattern of dark vermiculate markings over the head, numerous vertical bars across the flanks, and three pale transverse bands across the back, the broadest and most conspicuously demarcated being across the posterior nape and pectoral bases. The modified lateral-line system on the head lacks the cephalic canal ρ^1–ρ^2 (nomenclature of Sanzo[12]), but shows a seventh transverse sub-orbital row of sensory papillae, extension of the most posterior inferior segment of the sub-orbital series towards preopercular-mandibular row *e*, and approach or union in dorsal midline of occipital rows *o*. *Gobius quadrivittatus* was made the type of a new sub-genus (*Chromogobius*) of *Gobius* L. by De Buen[13]. Suitably expanded in definition to include another Mediterranean form (identified by me as *Gobius depressus* var. *zebrata* Kolombatovic), this taxon could well rank as a separate genus, for which *Relictogobius* Ptchelina 1939 becomes a junior subjective synonym of *Chromogobius* De Buen 1930.

Chromogobius quadrivittatus thus joins the increasing list of Mediterranean gobiids recognized as having penetrated into the Black Sea. These also now include *Gobius niger* L., *G. paganellus* L., *G. cobitis* Pallas (= *G. capito* C. & V.), *G. bucchichi* Steindachner, *G. ophiocephalus* Pallas, *Pomatoschistus minutus elongatus* (Canestrini), *P. leopardinus* (Nordmann) [*P. microps leopardinus* auctt., but specifically distinct from the true *P. microps* (Krøyer)], and *Aphia minuta* (Risso)[4,6,10,14]. All these species have in common an inshore habitat, several are known to tolerate brackish conditions, and all are widely distributed throughout the Mediterranean, with five out of nine extending outside the Mediterranean into the colder Atlantic Boreal region. In their euryhalinity and eurythermality (to be inferred from their wide geographical range), these gobies, when opportunity arose for colonization, would have been pre-adapted for life in the present Black Sea, with its broad temperature fluctuations in shallow waters and markedly lower salinity than is found in neighbouring parts of the Mediterranean[2]. In common with most of the other Mediterranean fishes which have permanently established themselves in the Black Sea, the gobies have not formed sub-species peculiar to the latter area, although local differentiation in the case of *Pomatoschistus leopardinus* is reported from the hypersaline lakes (41·8–44·3‰) at Eforia on the coast of Romania[15]. The presumably more stenotopic offshore gobiids of the Mediterranean (such as *Lesueurigobius*, *Deltentosteus*, *Buenia*, *Odondebuenia*, *Pomatoschistus* spp., and *Crystallogobius*, etc.) have not been recorded from the Black Sea. In parts of the Black Sea comparable to their known bathymetric range (25–300 m)[16], the values for temperature and salinity are appreciably less than those of the Mediterranean, and there is increasing deoxygenation and hydrogen sulphide pollution with depth[2,17], so that entry of these forms would seem to be effectively prevented by an array of ecological barriers. A reverse tendency for 'Caspian' gobiids to extend their range outside the Black Sea and its drainage area (including the Sea of Marmora) appears to be restricted to the brackish and freshwater *Proterorhinus marmoratus* (Pallas), found in rivers entering the northern Aegean Sea[4,5,18], and *Knipowitschia lencoranica* (Kessler) [= *Pomatoschistus caucasicus* (Kawrajsky) Berg], known from the mouth of the R. Yaila (Samothrace) and lagoons near Kavala, northern Greece (40° 56′ N., 24° 25′ E.)[14]. Spread of certain Caspian gobiids of Black Sea rivers into the Baltic Sea through Dnieper–Bug, Nieman, Dvina and Don–Volga–Ladoga canal connexions may also be expected[3,18].

I thank Dr. R. W. Hey, Geology Department, Cambridge, for advice on Ponto-Caspian successions. Useful accounts of Russian work on the biostratigraphy of Quaternary marine deposits in the Caspian, Black and Mediterranean Seas and also on the general correlation of local Quaternary sequences in Europe and the Ponto-Caspian region have recently appeared in English[19,20].

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LETTERS TO THE EDITOR

ASTROPHYSICS

Ionosphere, and X-ray Images of the Sun

Russell[1,2] has obtained X-ray photographs of the Sun at an epoch close to the recent minimum in solar activity which occurred late in 1964 and which preceded the present rising phase of the new solar cycle. The photographs were taken from a high-altitude rocket, and the quality and resolution are sufficiently good to allow limb brightening to be clearly distinguished from several small active regions that were present on the disk. The photographs seem to suggest that, in contrast to the brightening at the limbs, there was also some darkening at the north and south poles.

These directly made photographs provide at least qualitative confirmation of the solar brightness distribution derived by me[3] from ionospheric observations made during the previous solar minimum in 1954. This distribution was based on measurements of the changes in electron density, in the ionosphere, that were caused by the total solar eclipse of June 30, 1954. On the day of the eclipse, the Sun was exceptionally quiet and no active area of any importance was visible on the disk. Analysis of the ionospheric data led to the conclusion that, on this occasion, 82 per cent of the radiation could be attributed to an extended source which covered the entire disk and which was uniformly bright except for some darkening near the north and south poles. The remaining radiation (18 per cent) was attributed to limb brightening, although the slight asymmetry which was observed suggests that there may have been a very small active area near the west limb.

When ionospheric data are used to determine the brightness of the limbs relative to the uniform background, the degree of brightness which results depends on the value assumed for the effective recombination coefficient, α', in the expression:

$$J(t) = \left[\frac{1}{\alpha'}\frac{dNe}{dt} + Ne^2\right]\sec\chi$$

where $J(t)$ is proportional to the intensity of the incident radiation, Ne is the electron density at the peak of the E or $F1$ layer, and χ is the zenith angle of the Sun[4]. The accuracy of the calculated brightness distribution is limited mainly by the uncertainty in the value of α'.

It seems likely that the new X-ray photographs will give good quantitative information about the actual degree of limb brightening near the 1964 minimum, and it is probably reasonable to assume that the brightening during the 1954 minimum cannot have been very different. If this assumption is correct, it would be possible to use the 1954 ionospheric data, in conjunction with the 1964 value for limb brightening, in a new determination of α'. The result would be of considerable interest because the numerical value and the precise significance of α' have long been subjects for controversy.

Russell also refers to the presence of sources of X-radiation which extend outwards beyond the visible limb of the Sun, as predicted theoretically by Elwert[5]. A knowledge of the rapidity with which the intensity decreases with distance from the limb would allow estimates to be made of the amount of residual X-radiation which is present during the total phase of a solar eclipse. This information also could be used in the determination of α' from ionospheric data obtained during total eclipses, and its use for this purpose has been discussed on previous occasions[6,7].

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Radiation Balance of Jupiter and Saturn

It is frequently stated[1,2] that the radiation balance of Jupiter presents a significant problem in that the temperature derived for the cloud tops ($\sim 165°$ K) is considerably higher than the value which would be achieved by a rapidly rotating body having Jupiter's characteristics in simple equilibrium with solar radiation ($\sim 125°$ K). This discrepancy has been cited as possible evidence for thermal radiation from the interior of the planet; that is, it has been suggested that Jupiter might still be cooling down from an early, high-temperature phase[2]. The role of the atmosphere in maintaining the thermal balance has generally been disregarded since it has been assumed that the known constituents were all transparent in the wavelength region of importance (20–40μ). The situation for Saturn is very similar; the temperatures are simply somewhat lower. A number of recent findings when considered together appear to require a revision of these ideas.

Trafton[3] has pointed out that the pressure-induced dipole absorption of H_2 may produce significant absorption in the case of Jupiter. He finds that temperatures of the order of 147° K are not difficult to maintain by an atmospheric greenhouse effect depending only on this absorber. Owen[4] has reviewed a number of measurements of the temperature of Jupiter and has analysed one of the methane bands in the planet's spectrum to obtain a value of $200° \pm 25°$ K. The conclusion of this investigation was that there appeared to be strong evidence for temperatures in the range 175°–200° K in and above the cloud layer which is envisaged as considerably more complex than a simple flat surface. It thus seems necessary to invoke another absorber if the atmosphere is to provide the necessary opacity.

The way out of this dilemma appears to be provided by some recent laboratory studies of ammonia. Walsh[5] has obtained spectra of ammonia in the region 20–35μ at a number of path-lengths, with the gas subjected to various degrees of pressure broadening by N_2. A series of his tracings for an equivalent path-length of about 12 m atm. of ammonia at three different pressures of N_2 is reproduced in Fig. 1. It is evident that, even with no broadening by N_2, the transparency in this region is very low. In Jupiter's atmosphere, of course, the temperature will be lower than the laboratory value, so the population in the higher rotational levels (which are responsible for the absorption shown in Fig. 1) will be diminished. On the other hand, the atmospheric pressure within the region of the clouds is almost certainly in excess of 76 cm mercury and, while the broadening gases will be H_2 and He instead of N_2, the susceptibility of ammonia to pressure broadening is so great that at the expected pressures the absorption

should lie within the range displayed by the three curves in Fig. 1. Whether this will indeed be sufficient to produce the required greenhouse effect can only be demonstrated by a more careful treatment which includes the absorption at other wave-lengths. The spectrum of appropriately large amounts of methane should also be examined to see whether the band at 44μ may be a significant source of opacity.

Saturn presents a rather different problem. Following Dunham[6], it is generally assumed that Saturn's atmosphere also contains ammonia. However, recent examinations of the spectrum of this planet have failed to reveal the absorption bands of this gas[7], and an alternative explanation for Dunham's identification of ammonia in terms of an overlapping methane band has been adduced[8]. Furthermore, a recent temperature measurement by Low[9] at a wave-length of 10μ has led to a value of 93° K, well below the earlier results of ~130° K. At 90° K, the vapour pressure of ammonia is only 10^{-4}μ mercury, suggesting that its spectroscopic detection is extremely unlikely. Hence the low temperature and the absence of ammonia absorption are consistent.

It thus seems highly probable that the 93° temperature refers to some level of the atmosphere within the cloud layer, that is, the thermal radiation measured at 10μ is coming from cloud particles rather than from the ν_2 absorption band of ammonia as in the case of Jupiter. (There may be some radiation from higher rotational lines in the nearest strong methane band at 7·7μ.) A model for the detailed structure and composition of the cloud layer will have to await additional observations, but it seems likely that the absence of convective phenomena driven by the large latent heat of sublimation of ammonia (441 cal/g mole at 198° K) may be responsible for the greater visual uniformity of the disk of Saturn compared with that of Jupiter. While it is inevitable that clouds consisting of ammonia crystals will form at some atmospheric level, the low temperature of the upper atmosphere of Saturn also permits the deposition of methane, suggesting a possible explanation for the difference in the polarization of Jupiter and Saturn reported by Dollfus[10]. In this view, the atmosphere above the level of visual opacity in the case of Jupiter is charged with ammonia crystals, while in the much colder atmosphere of Saturn these are replaced by crystals of methane. The difference in the polarization of the two planets could then arise from the difference in the index of refraction of the two kinds of crystals or a difference in their mean size.

The consistency established by the low temperature and absence of ammonia in the atmosphere of Saturn has interesting implications for the foregoing argument. Saturn's atmosphere is known to contain methane and hydrogen in somewhat greater amounts than the atmosphere of Jupiter. (The increase is due to the lower level of the cloud layer in the atmosphere of Saturn.) Yet the measured temperature is very close to the radiation equilibrium value (~90° K), and apparently refers to a region in the atmosphere very close to the visually observed cloud layer. In other words, in the absence of a detectable quantity of gaseous ammonia, a Jovian-type atmosphere does not seem to have sufficient opacity to produce a large greenhouse effect. Thus either Jupiter is, in fact, still cooling down (while Saturn is not), or it is the presence of a substantial amount of ammonia in its atmosphere which leads to the observed high temperatures. The laboratory evidence presented in Fig. 1 suggests that the latter alternative must be seriously considered before resorting to the internal heat source suggested by the former.

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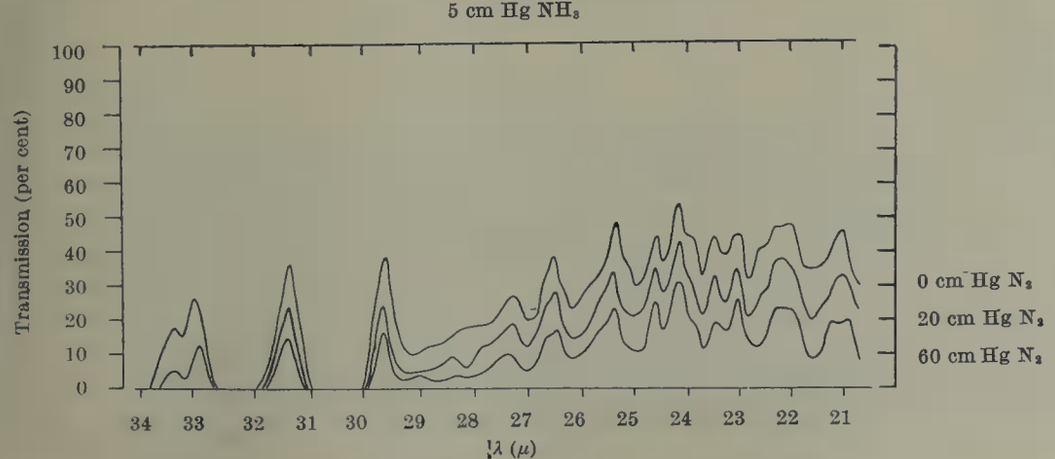

Fig. 1. Absorption spectra of ammonia subjected to pressure broadening by nitrogen (ref. 5)

GEOPHYSICS

Daily Variation of Cosmic Rays

One of the interesting properties of cosmic radiation of intermediate energies, 1–100 GeV, is the daily variation of intensity measured at the Earth. This variation, of average amplitude ~0·3–0·4 per cent, appears largely diurnal in character although harmonic analysis reveals a semidiurnal component. The maximum intensity occurs, on the average, about 1700 L.T. although the phase can vary considerably.

An understanding of the mechanism producing the variation has increased in the past few years with the discovery of the solar wind, the development of a model of the large-scale magnetic field configuration in the inner solar system, and the use of computers to achieve detailed information about the trajectories of charged particles in the geomagnetic field. Several theories have been put forward in recent months to account for the observed anisotropy in terms of the (overtaking) streaming of the cosmic ray gas parallel to the Earth's motion around the Sun. On the other hand, Sarabhai *et al.*[1] have suggested that part of the daily variation is due to removal of particles from the morning side of the Earth due to scattering by magnetic irregularities. This communication proposes an alternative mechanism for such removal.

Several investigators have calculated particle trajectories under the influence of the Earth's magnetic field to ascertain 'asymptotic' directions of such particles, as a function of rigidity, outside this influence. By projecting a negative particle from the Earth (re-creating the history of a positive particle arriving at the Earth) and following its trajectory until the path leaves the

magnetosphere, orbit calculations have been made in detail for protons entering this region of influence of the Earth. Allowing for the energy spread of primary particles capable of contributing effectively to neutron monitor counting rates, and for atmospheric effects, protons may enter the magnetosphere within an appreciable cone of directions prior to detection on the Earth at any location. The cone will, however, be restricted to equatorial latitudes for low and middle latitude detectors.

Outside the magnetosphere, however, the magnetic field has a value of about 3 or 4γ, and is usually considered to be oriented in the basic pattern of an Archimedes spiral centred on the Sun. When the cone looks up the Archimedes spiral, most of the detected primaries will have mirrored between the Sun and the Earth, assuming that the Alfven guiding-centre approximation is valid and ignoring magnetic irregularities in the spiral. Cosmic ray primaries approaching the Earth from the antisolar direction up the spiral will be largely isotropically distributed in direction. (We should, perhaps, emphasize that in this model the majority of particles detected at the Earth by neutron monitors are confined to the spiral arm.) Passing the Earth, those the pitch angles of which are large will mirror near the Earth and then pass outwards along the spiral. For smaller pitch angles the mirror points will be closer to the Sun. For very small pitch angles, however, the mirror points will be in the Sun and the particles will be lost. Using the subscripts E and S for values at the Earth and Sun respectively, the critical value for the pitch angle α_E is given, assuming the conservation of magnetic moment, by:

$$\frac{\sin^2\alpha_E}{\sin^2\alpha_S} = \frac{B_E}{B_S}$$

that is, $\sin\alpha_E = (B_E/B_S)^{1/2} \approx 5\times10^{3}$ assuming $B_r \propto$(distance from Sun)$^{-2}$, that is, $\alpha_E \approx 5\times10^{-3}$ rad. Assuming an isotropic pitch angle distribution at the Earth, the number of particles absorbed by the Sun will be:

$$\Delta N = \frac{N}{4\pi}\int_0^{2\pi} d\varphi \int_0^{\alpha_E} \sin\theta\, d\theta \approx 10^{-5}\, N$$

where N is the number density of particles. The free space anisotropy should therefore be of the order of 0·001 per cent. Neutron monitors, however, detect primaries from restricted angle of approach. For Sulphur Mountain the effective solid angle is about 2×10^{-2} sterad. Hence the detected flux:

$$F \approx \frac{2\times10^{-2}}{4\pi}\cdot NV \approx 1{\cdot}7\times10^{-3}\, NV$$

where V is the velocity.

Thus $\Delta F/F = \Delta N\cdot V/F \approx 0{\cdot}6$ per cent when the asymptotic direction of approach coincides with the Sun–Earth field line. This value, however, overestimates the magnitude of the anisotropy since the particles, of differing rigidities, absorbed by the Sun would not have contributed with equal effectiveness to the directional response of the detector. On the other hand, if B_r should vary more slowly than $1/r^2$, the effect will be enhanced.

The effect of the cosmic-ray gas streaming past the Earth due to co-rotation of the gas with the Sun has been estimated by Axford[2] and Parker[3] to be of the order of 0·4–0·7 per cent. The predicted minimum due to this effect is at 0600 L.T. The solar absorption effect will, however, be directed up the spiral, 45° west of the Earth–Sun line. The resultant will therefore be the vector sum of the two effects. Figs. 1*a* and *b* show the frequency of occurrence of the experimentally observed anisotropies on polar diagrams for 1964 for selected days at Sulphur Mountain and Calgary respectively (counting rates of 10^6 and $2{\cdot}5\times10^5$/h respectively). Selection of the data was based only on internal consistency between the two monitors in the shapes of the sums of the first and second harmonics of the data for each monitor for each day. Data from 154 days are included. It is clear from these plots that the minimum direction is centred on 60° west of the Earth–Sun line. The averaged amplitudes of the minimum for the data taken together are $0{\cdot}56\pm0{\cdot}01$ per cent and $0{\cdot}56\pm0{\cdot}01$ per cent for Sulphur Mountain and Calgary respectively. Both direction and magnitude of the observed minimum are in fair agreement with the vector sum of the two effects considered, taking the lower value of 0·4 per cent for the streaming mechanism.

The absorption mechanism suggested by the authors is restricted by the following considerations:

(1) The gyroradii of the participating particles must be less than the scale size of irregularities in the magnetic field. This being so, the guiding centre approximation remains valid. When the gyroradii are of the same magnitude as the irregularities, however, the particles are scattered[4] by the latter, and the fraction that reach the Sun will be small.

The gyroradii of protons the pitch angles of which are $\lesssim 5\times10^{-3}$ radians in a field $B_E = 4\gamma$ and the energies of which are 1, 10 and 100 GeV are less than $5{\cdot}5\times10^3$, $4{\cdot}5\times10^4$ and $4{\cdot}0\times10^5$ km respectively.

Assuming the spiral field suggested by Parker[3] and the constancy of $(\sin^2\alpha)/B$, it follows that the gyroradius of a proton decreases almost linearly with decreasing distance from the Sun. Measurements by *Explorer*

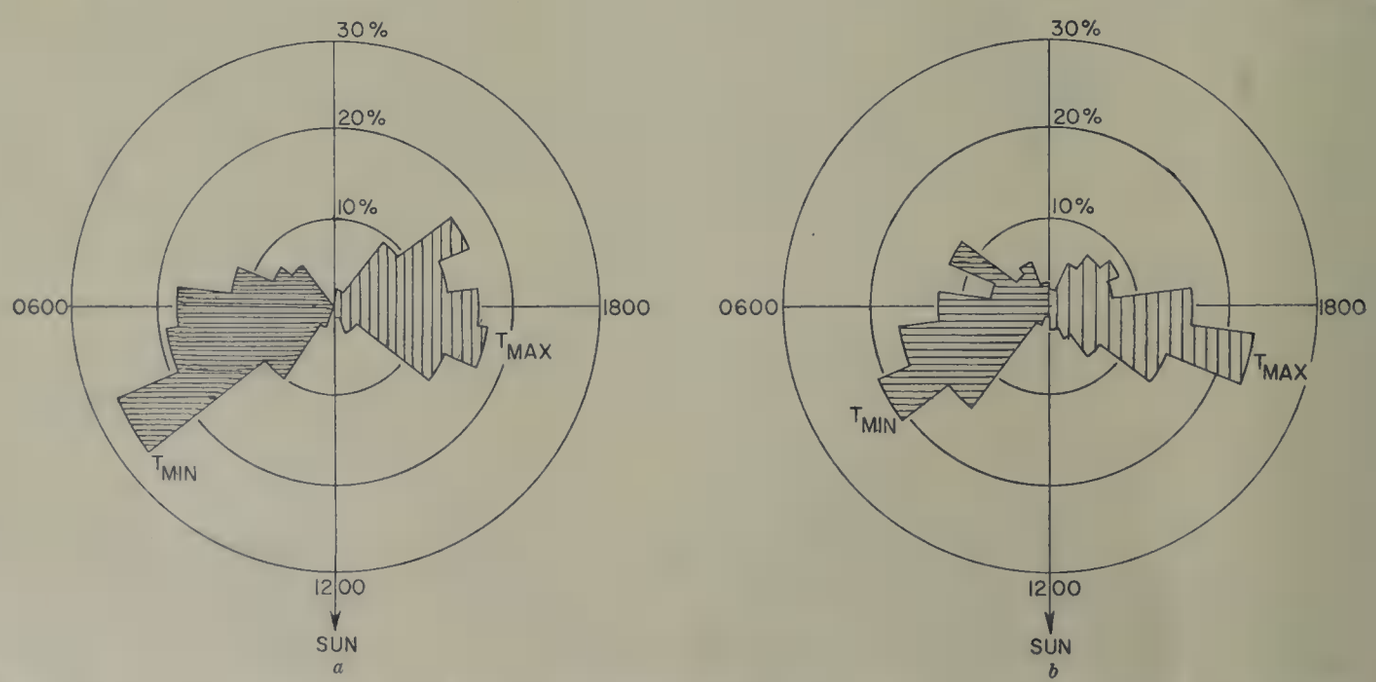

Fig. 1. Direction-frequency plot of T_{max} and T_{min} for the daily variation in neutron intensity for 154 days during 1964 at (*a*) Sulphur Mountain and (*b*) Calgary, Canada

XVIII[5] indicate periods in which magnetic irregularities of 10^5–10^8 km exist. If 10^5 km is the minimum extent of the fluctuations, then the mechanism will be operative and will be independent of energy. Should smaller irregularities occur an energy dependence would be expected.

(2) The direction of minimum intensity will be that of the field lines at any instant, and since their direction changes over periods of a few minutes to a few hours $\left[\frac{10^5 \sim 10^7 \text{ km}}{450 \text{ km/sec}}\right]$ there will be a broadening of the minimum and perhaps a difference in the recorded time of minimum even for stations whose cones of acceptance do not greatly differ.

(3) Changes in direction of the 'average' spiral field on a day-to-day basis will be responsible for part of the spread in direction of T_{min} as observed in Figs. 1*a* and *b*. Such fluctuations would be related to changes in solar wind velocity.

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Frequency Spectrum of Doppler-shifted Whistler-mode Signals

To further our knowledge of very-low-frequency signals received via the whistler mode of propagation we have constructed a 25-channel spectrum analyser. Other work[1,2] has shown the existence of apparent Doppler shifts on these signals of up to two or three parts in 10^5 of the carrier frequency; but the equipment used did not allow the variations of doppler shift with time to be resolved to better than 30 min. It sometimes appeared that there could be large positive and negative shifts occurring within a single 30-min period.

The new equipment consists of 25 filters, of the coherent detector–low pass amplifier type, in which the filter is tuned to the reference frequency supplied to the coherent detector and the band-width is determined by the low-pass amplifier. Use of this type of circuitry reduces the problem of the alignment of a bank of closely spaced filters to one of frequency synthesis, which in this case is done electro-mechanically. One filter is at the nominal carrier frequency and the others are disposed about this, 12 above and 12 below at a nominal spacing of 2 parts in 10^6 of the carrier frequency. Each channel has a band-width of approximately 0·5 parts in 10^6.

In the short time that the equipment has been operating whistler-mode activity has been seen fairly frequently at off-sets of several parts in 10^6. Occasionally events such as those shown in Fig. 1 are seen in which the Doppler shifts are oscillatory in nature. This is shown more clearly in Fig. 2 where the median frequency shift is plotted as a function of time. It is probable that it was events of this nature

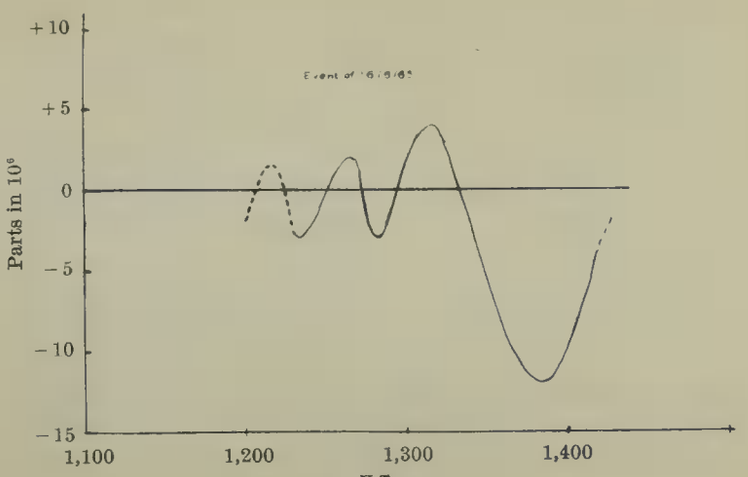

Fig. 2

which, when recorded on the original equipment, indicated the possibility of simultaneous positive and negative Doppler shifts.

The signals in this case are from station *NPG* at Seattle on a frequency of 18·6 kc/s. It will be noticed that there is a short period of 'key-up' followed by a period of 'key-down' just prior to the beginning of each hour. The loop aerial was turned to obtain a minimum of the direct or ionospheric signal, this being done to limit the spill-over of this component into the filters adjacent to the centre frequency. The large-amplitude slow-period beat on the centre channel is the result of a perfect null not being obtained. The period of the beat indicates the difference between the carrier frequency, as received, and the local standard.

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GEOCHEMISTRY

Radioactive Elements in the Continental Crust

The radioactive elements are particularly useful as indicators of the chemical composition of the deeper parts of the crust. Thorium, uranium, and potassium show a strong concentration towards the surface of the Earth and are enriched in the continental crust. The abundance of thorium, uranium and potassium can be estimated by two independent methods; a physical method based on heat-flow data, and a geochemical method based on geological premises. These estimates differ by a factor of two (Table 1). The estimates for the heat-flow data are from Clark and Ringwood[1], and are based on a 37-km-thick two-layer crust. The upper layer is 16 km thick, resting on a layer 21 km thick. Calcu-

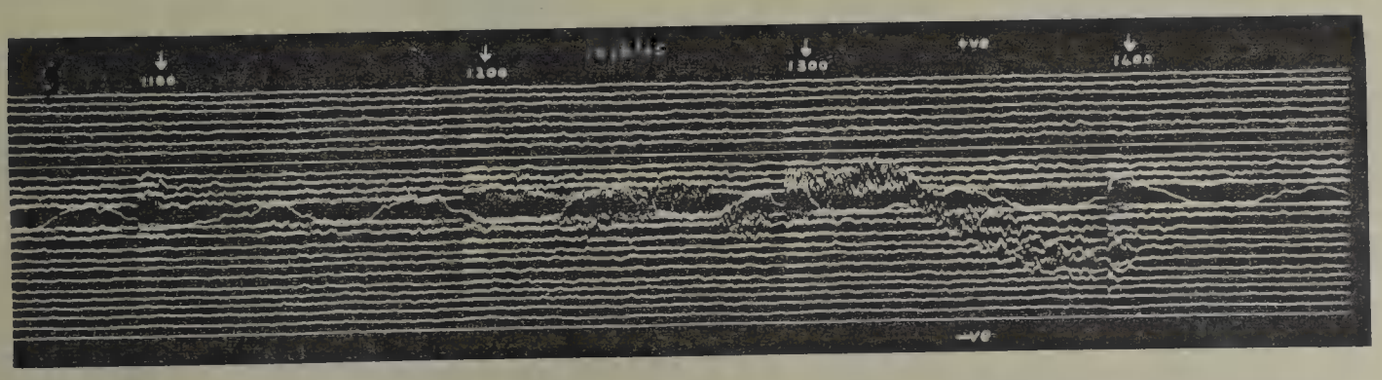

Fig. 1

Table 1. Estimates of Thorium, Uranium and Potassium Abundances in Regions of the Continental Crust

Th (p.p.m.)	U (p.p.m.)	K (per cent)	Ref.
			Geochemical estimates
11·4 ± 2	3·0 ± 0·6		Adams *et al.*[12]; geochemical balance; abundance in crust exposed to weathering
10·0	2·8	2·6	Heier and Rogers[13]; correlation between Th, U, and K in magmatic rocks; K figure adopted from MacDonald[14] (continental crust)
9·6	2·7	2·09	Taylor[9]; 1 : 1 ratio of basalt and granite (continental crust)
9·0	2·3	2·55	Clark *et al.*[2]; median of granodiorites
8·8	2·2	2·35	1 : 1 ratio of mafic and felsic rocks (data from Clark *et al.*[2])
5·3	1·4	1·5	1 : 1 ratio of basic rocks and granodiorite (data from Clark *et al.*[2])
			Estimates from heat flow
4·6	1·12	1·84	Clark and Ringwood[1]; surface heat flow 1·5 μcal/cm² sec; average of 37 km crust
3·2	0·81	1·31	Surface heat flow 1·2 μcal/cm² sec
2·5	0·64	1·05	Surface heat flow 1·0 μcal/cm² sec

Table 2. Estimates of Thorium, Uranium and Potassium Abundances in Different Regions of the Continental Crust

Abundances derived from heat flow data (after Clark and Ringwood (ref. 1))

	Surface heat flow (μcal/cm² sec)	Depth interval (km)	U (p.p.m.)	Th (p.p.m.)	K (per cent)
Shield	1·0	0–16	1·00	4·00	1·63
		16–37	0·37	1·48	0·61
Continental	1·2	0–16	1·32	5·28	2·15
		16–37	0·42	1·68	0·69
Continental	1·5	0–16	1·87	7·48	3·05
		16–37	0·57	2·28	0·93

lations of the concentrations of thorium, uranium and potassium within each layer for different heat flows are given in Table 2. It is noteworthy that the concentrations of the radioactive elements in the 16-km layer in regions with high heat flow are comparable with the geochemical estimates made on the basis of the new abundance data by Clark *et al.*[2]. The concentrations calculated for the lower layer are similar to those in basalts. This could indicate that this layer has a basaltic composition. However, metamorphic processes, including generation of granite magma, and the cycling of elements within the crust could also account for the low concentrations of radioactive elements in the deep crust[3–5]. These processes would result in a more mafic average composition of the lower crust as compared with the higher crust[3–8]. There is also some indication[5] that high-grade metamorphic rocks, especially those of granulite facies, have lower concentrations of thorium and uranium than magmatic rocks of comparable chemical composition, so that the upward concentration of thorium and uranium in the crust could be more marked than that of potassium.

Crustal abundances in general are derived from premises similar to those used to obtained the geochemical estimates of uranium and thorium in Table 1. It is possible, therefore, that we are at present overestimating many of the volatile and 'granitophile' elements in the crust, and that the average continental crust is more mafic than present estimates[8,9]. It is worth noting that Clarke[10] and Goldschmidt[11] restricted their estimates to the composition of a 10-mile-thick upper crust.

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PHYSICS

Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides

To obtain the integrated absorption strength of a narrow spectral line by direct spectrophotometric measurement high resolution is necessary and the resultant profile must be corrected for finite instrumental resolution. As an alternative, dispersion data at much lower resolution may be used to obtain line strengths, and we report some measurements on a few of the low J rotational transitions in the halogen halides.

Table 1. Sub-millimetre Rotational Line Strengths in the Hydrogen Halides

Rotational transition $J \rightarrow (J+1)$	HCl k_i (cm⁻¹)	HCl A_i^0 (cm⁻² atm⁻¹)	HBr k_i (cm⁻¹)	HBr A_i^0 (cm⁻² atm⁻¹)	HI k_i (cm⁻¹)	HI A_i^0 (cm⁻² atm⁻¹)
0→1	20·88	1·73 ± 0·34	16·70	0·24 ± 0·02	—	—
1→2	41·76	8·25 ± 0·49	33·40	2·13 ± 0·05	25·70	0·42 ± 0·03
2→3	—	—	50·10	5·39 ± 0·30	38·54	1·10 ± 0·11
3→4	—	—	—	—	51·36	2·72 ± 0·09

We have used the method of Fourier refractometry[1,2] to obtain the refractive index of gaseous HCl, HBr and HI in the region 10–55 cm⁻¹ at a resolution of 2 cm⁻¹. The gases were carefully dried before introduction into a cell placed in one arm of a Michelson interferometer. Dispersive interferograms were recorded for various gas pressures up to about 500 mm mercury at temperatures near 293° K, and after Fourier transformation the difference $n(k) - \bar{n}$ between the refractive index $n(k)$ at wave-number k and the mean $\bar{n}$ over the radiation band was calculated[2] from:

$$n(k) - \bar{n} = \frac{1}{4\pi t k}\left\{\arctan\frac{Q(k)}{P(k)} + \pi m\right\} \qquad (1)$$

where $P(k)$ and $Q(k)$ are respectively the cosine and sine Fourier transforms of the interferogram function, m is an integer, and t is the cell length (20·3 cm). A typical set of results for HCl at three pressures is given in Fig. 1, in which the anomalous dispersion associated with the lines $J = 0 \rightarrow 1$ and $J = 1 \rightarrow 2$ is seen. The data were reduced to 760 mm mercury pressure and 300° K temperature using a standard form[2,3], and from these the strength per atmosphere A_i^0 of a line at wave-number k_i was calculated using the relation[4]:

$$A_i^0 = \frac{2\pi^2}{f_i(k', k'')}\left\{n_i^0(k') - n_i^0(k'')\right\} - \sum_{j \neq i}\frac{A_j^0 f_j(k', k'')}{f_i(k', k'')} \qquad (2)$$

between the refractive index difference at two wave-numbers k', $k''(k' < k_i < k'')$ and the absorption strength. We have written:

$$f_i(k', k'') = \left\{\frac{1}{k_i^2 - k'^2} + \frac{1}{k^2 - k''^2}\right\} \qquad (3)$$

The experimental index values determined near any given transition k_i include the significant contributions of

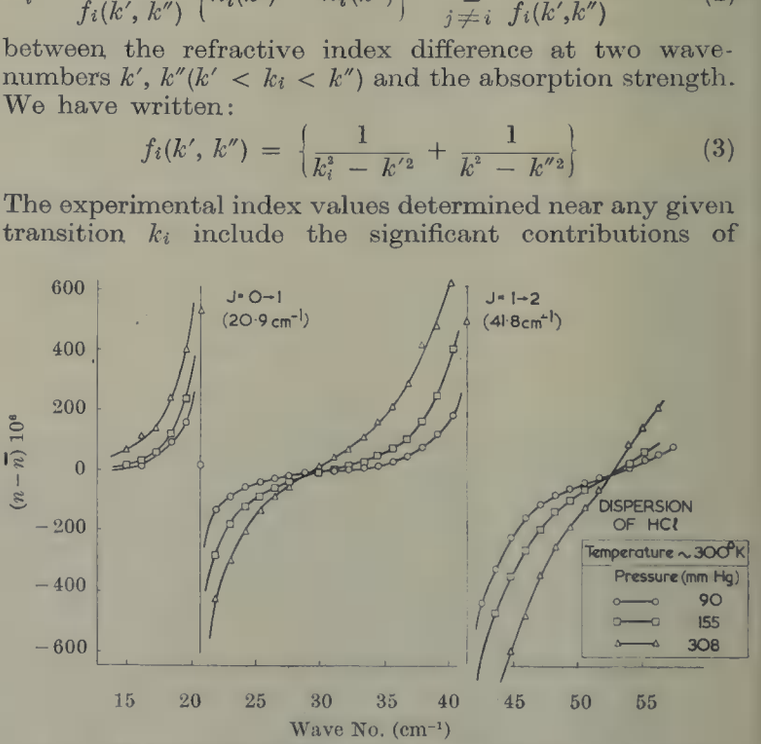

Fig. 1. The sub-millimetre dispersion of gaseous HCl in the region of the $J = 0 \rightarrow 1$ and $J = 1 \rightarrow 2$ rotational lines. Each point represents the average of two independent measurements made at the pressure stated, and the curves join the experimental data points. Cell-length is 20·3 cm

neighbouring transitions k_j and account is taken of these by using the experimental refractive index data in equation (2) and including the term under the summation. The various A_i^0, A_j^0 are found by an iterative method since they are all initially unknown. Details of the experimental method and calculation will be given elsewhere. Using our index data and some recently published values of the rotational constants[5] to calculate the k_i in equation (2), we find the values for the line strengths given in Table 1.

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CRYSTALLOGRAPHY

X-ray Studies on Highly Pure Magnesium Oxide

High-purity (99·999 per cent) magnesium oxide was prepared by the thermal decomposition of pure magnesium oxalate dihydrate. Details of the preparation, analyses and thermal decomposition of the magnesium oxalate have been given elsewhere[1,2]. Prior to the X-ray examination, the magnesium oxide was recrystallized by firing as a pressed disk at 1,500° C for 24 h. X-ray powder diffraction data were obtained by means of a 19 cm diameter camera using copper K_α-radiation with a nickel filter, and standard procedures. The observed spacings are given in Table 1.

Table 1. X-ray Data for Pure MgO

hkl	*I/Io*	*d* (Å)
111	4	2·433
200	10	2·108
220	10	1·4899
311	4	1·2695
222	6	1·2155
400	3	1·0529
	2	1·0529
331	4	0·9662
	1	0·9661
420	4	0·9418
	2	0·9418
422	4	0·8594
	2	0·8594
511	2	0·8103
	1	0·8104

Table 2. Comparison of the Lattice Constants of MgO

Material	Lattice constant (Å)
MgO, data of Swanson and Tatge (ref. 4)	4·213
MgO, present study	4·2119 ± 0·0005

The accurate lattice constant of the cubic magnesium oxide was determined by the method of Nelson and Riley[3], using sodium chloride as an internal standard. The result obtained is given in Table 2 together with the value given by Swanson and Tatge[4], for comparison.

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CHEMISTRY

Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces

Measurements of the differential capacitance of the electrical double-layer at an LiCl–KCl eutectic melt/platinum interface at 450° C have been made by Laitinen and Roe[1] over a range of potentials from −0·34 V to −2·11 V versus a Pt/Pt^{2+} (1 *M*) reference electrode. Their curve of capacitance versus potential can be roughly divided into two regions: an anodic region of comparatively high specific capacitance (~100 μF cm^{-2}) and a cathodic region of low specific capacitance (~50 μF cm^{-2}), the transition between them occurring at approximately −0·8 V to −1·0 V. The high values of capacitance were attributed[1,2] to the specific adsorption of chloride ions at potentials which are presumably anodic to the point of zero charge of this interface.

Double-layer capacitance versus potential curves for other metal/melt interfaces are quite unlike the previously published curves for platinum[1,2,4] in that they are symmetrical about a well-defined minimum at the potential of zero charge (silver, thallium and tin are exceptions to this rule in that they exhibit a step in the cathodic branch[5]). Although it appears that the halide ions are surface active, because, for example, the addition of iodide ions to a chloride melt produces an increase in double-layer capacitance[6], the increase is the same in both the anodic and cathodic branches of the curve for a given $|\varepsilon|$ (the magnitude of the rational potential).

The present work suggests that the double-layer capacitance of a platinum/melt interface is dependent on the well-known existence of oxide layers on platinum surfaces[7], and on the specific adsorption of platinum ions at concentrations determined by the redox potential of the melt.

The capacitance of the double-layer at a flame-polished, platinum hemisphere microelectrode under certain well-defined conditions (see following) falls to a minimum at approximately −0·8 V versus a Pt/Pt^{2+} (1 *M*) reference electrode (Fig. 1, curve *a*). In the region from −0·6 V to −0·8 V the capacitance varies with time so that it is

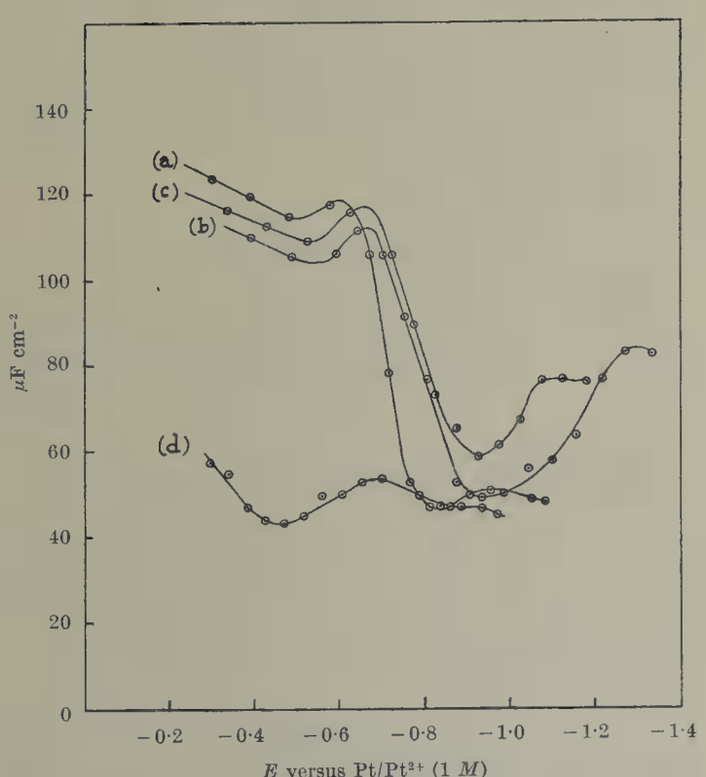

Fig. 1. The differential double-layer capacitance at platinum microelectrode/LiCl–KCl eutectic melt interfaces at 450° C. (*a*) Pure melt; (*b*) ~10^{-6} *M* Pt^{2+}; (*c*) ~10^{-5} *M* Pt^{2+}; (*d*) pure melt

necessary to allow up to 10 min for the system to come to equilibrium at each value of potential. If this procedure is not adopted, the curve becomes less well-defined and shows a marked similarity to the curves obtained by other workers[1,2,4], provided that the potential is changed in a cathodic direction. If the potential is changed in an anodic direction and time is not allowed for the system to come to equilibrium, the higher values of capacitance are never attained (Fig. 1, curve *d*). Under equilibrium conditions curve (*a*) in Fig. 1 can be reproduced without any difficulty whatever the direction of potential change; provided that the potential of the platinum electrode is not taken too far into the cathodic region, where there is risk of surface modification by, for example, the deposition of alkali metal.

The slow change of capacitance with time at potentials in the range $-0{\cdot}6$ V to $-0{\cdot}8$ V can be attributed to the desorption of chemisorbed oxygen atoms or the cathodic stripping of a stoichiometric oxide from the platinum surface. This process is reversed as the electrode potential is returned through the $-0{\cdot}8$ V to $-0{\cdot}6$ V region, because of the presence of low concentrations of oxide ions even in highly purified melts in glass apparatus[8] (purified by vacuum pre-electrolysis and filtration[9]). It is significant that the adsorption process is slower than the desorption process, reflecting not only the irreversibility of the process but also the inaccessibility of the oxide ions present at low concentration in the melt.

If the platinum surface is completely stripped over the range $-0{\cdot}6$ V to $-0{\cdot}8$ V, then this latter value (the point of minimum capacitance) is the zero charge potential for the system (the average value calculated from a number of experiments is $-0{\cdot}83 \pm 0{\cdot}02$ V versus the Pt/Pt^{2+} (1 *M*) reference electrode). This value agrees rather well with the proposition that the difference between the standard electrode potential and the potential of zero charge of an M/M^{2+} couple is in the range of 0·5 V–0·9 V[10].

The addition of very small quantities of platinum ions to the melt by anodic dissolution of a platinum counter-electrode produces a number of changes in the capacitance versus potential curve (Fig. 1, curves *b* and *c*), the most interesting of which is the formation of a better defined minimum and the shift of this minimum to more cathodic potentials. This suggests that platinum ions are adsorbed on to the electrode surface as negatively charged species; perhaps as a square-planer $PtCl_4^{2-}$ complex ion, which is particularly suited to adsorption on a plane surface and can lead to a more compact structure for the double-layer. The minimum capacitance in curves (*b*) and (*c*) is indeed increased over that of the pure melt even for the very lowest concentration of platinum ions and this is typical of an adsorption phenomenon. This effect could also arise from a change in the electrode surface following the electrodeposition of platinum metal. However, curve (*a*) in Fig. 2 indicates that this is not so. This curve was obtained with the previously used microelectrode introduced into a fresh melt without prior surface treatment. The minimum capacitance was reproduced within the accuracy of the method (± 3 per cent) and the addition of platinum ions (in somewhat higher concentrations than before) produced the characteristic increase at the minimum together with the cathodic shift (Fig. 2, curve *b*).

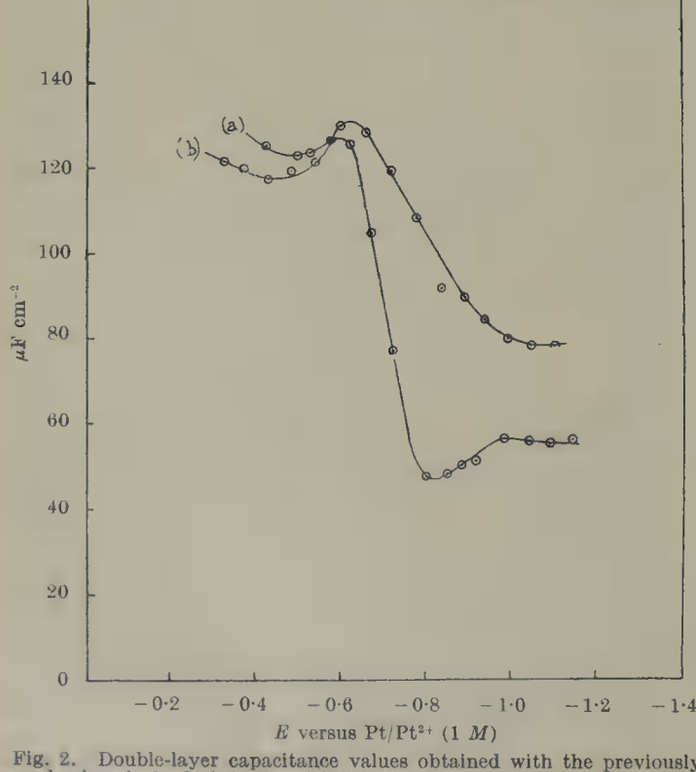

Fig. 2. Double-layer capacitance values obtained with the previously used microelectrode introduced into a fresh melt without prior surface treatment. (*a*) Pure melt; (*b*) $\sim 10^{-4}$ *M* Pt^{2+}

A method has been developed to make these experiments possible, the details of which are published elsewhere[10]. A fast rise-time (10^{-8} sec) constant current pulse is applied to the electrode system, and measurements are made on the resulting over-potential/time transient before the faradaic process interferes, that is, within 2×10^{-7} sec of the initiation of the pulse[11]. This method[12] has been modified[10] in the present work to enable measurements to be made at electrode potentials other than the equilibrium redox potential of the system. It is possible, therefore, to measure a true double-layer capacitance (free from pseudocapacitance) even in the presence of a fast electron transfer process, and hence detect the effects of the adsorption of the electroactive species.

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Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids

Although the mechanism of recoil atom reactions in solids has been studied by a number of authors, some important problems still remain unsolved. For example, thermal and hot processes were discriminated in the recoil atom reactions in the gaseous or liquid system[1], but this is not clear in solids. Harbottle and Sutin[2] have proposed a concept of chemical reactions in the 'hot-zone'; however, this concept was somewhat ambiguous from the point of view of the presence of thermal and hot processes in the 'hot-zone'. In the course of studying hot-atom chemistry of copper-64 recoil atoms in α- and β-copper phthalocyanines, we have found evidence of thermal and hot processes in the 'hot-zone' in solids.

The hot-atom chemistry of copper phthalocyanine has been investigated by Herr and Götte[3], while the effects of crystal structure on the initial retention and on the thermal annealing rate have been investigated by Cook[4] and by ourselves[5].

Purified α- and β-copper phthalocyanines were checked by the X-ray diffraction method. Neutron irradiation of the target materials was carried out in a nuclear reactor *TRIGA* Mark II at Rikkyo University (neutron flux: 5×10^{11} n/cm²/sec). An irradiated sample was dissolved in cooled sulphuric acid (~ −20° C), and the solution poured into ice water to precipitate copper phthalocyanine. Afterwards the precipitate was filtered, and the precipitate and the filtrate separately counted by means of a Baird-Atomic single channel γ-ray spectrometer (crystal: NaI, 1·75 in. × 2 in.).

The relationship between the initial retention and irradiation temperature is shown in Fig. 1. To avoid any effects of thermal and radiation annealing during irradiation, all retention values have been corrected for irradiation time $t = 0$. A remarkable difference between α- and β-crystals can be seen in Fig. 1. The initial retention in β-crystals at low temperature R_{β_0} is much higher than that in α-crystals R_{α_0}. The shape of the curve of β-crystals is different from that of α-crystals. This sensitivity of the initial retention in β-crystals seems to be closely related to the rate of increase of the retention on annealing. Table 1 shows initial annealing rates in α- and β-crystals at various temperatures. A remarkable difference between α- and β-crystals can again be seen.

Table 1. Comparison of Initial Annealing Rates in α- and β-Crystals

Annealing temperature	Initial annealing rate (per 40 min) α	β	α/β
87° C	0·25%	4·0%	1/16
139° C	1·0	20	1/20
198° C	3·4	50	1/15
		Mean	1/17

According to Harbottle's model[2], hot-zone reactions quench in a very short time after the recoil event, and therefore they are not completed in a 'hot-zone', but continue on thermal annealing. In other words, a reaction in a 'hot-zone' will be succeeded by substantially the same kind of reaction at the recoil site on thermal annealing. If this is true, the relationship of annealing rate between α- and β-crystals must hold good for that of 'hot-zone' reactions between α- and β-crystals (at least in the thermal range of the 'hot-zone'). On this assumption, we can simply go back to 'hot-zone' reactions from thermal annealing as is shown schematically in Fig. 2(*a*). At the crossing-point of two lines the quantity R_h (= 5·0 per cent) can be obtained. R_h represents reactions which complete in the 'hot-zone' and is considered to be contributed by hot processes. In this way we can estimate the percentage of thermal processes and hot processes. Fig. 2(*b*) shows the results obtained. The apparent insensitivity of the retention in α-crystals to temperature, and the sensitivity of the retention in β-crystals to temperature, can be easily

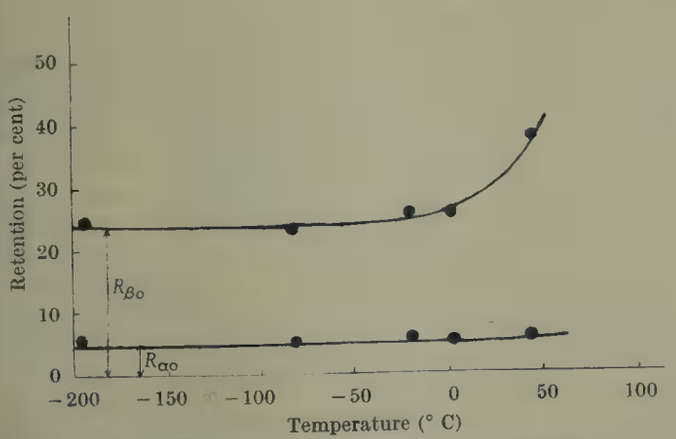

Fig. 1. Relationship between initial retention and irradiation temperature

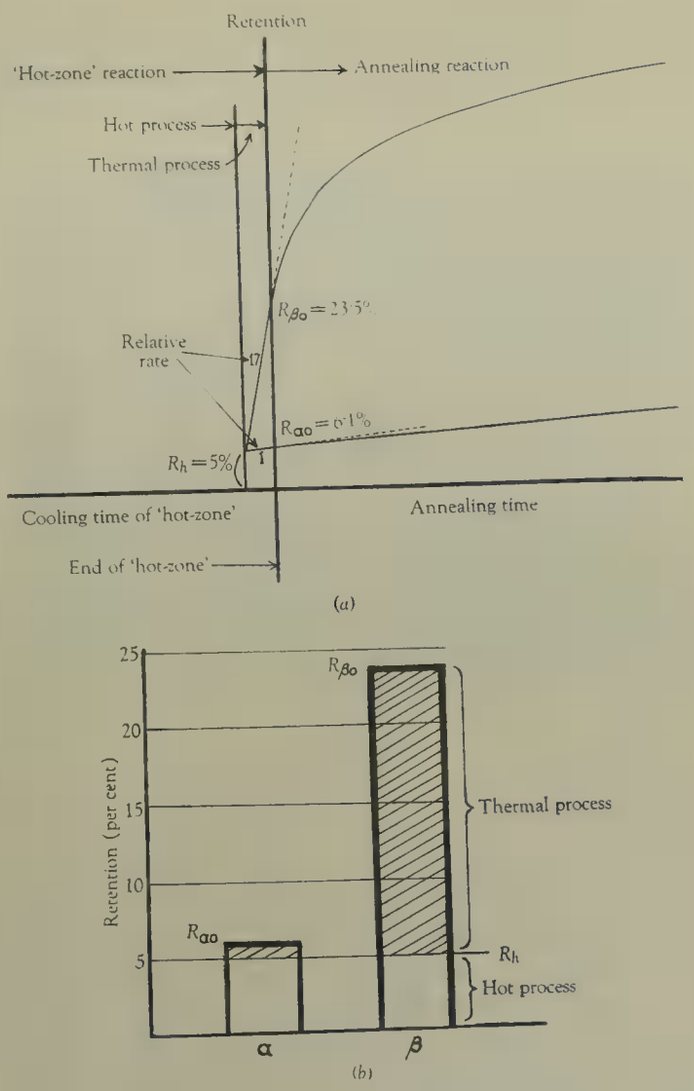

Fig. 2. Estimation of thermal and hot processes in the 'hot-zone'. (*a*) Methods of analysis. (*b*) Results

understood by considering the extent of the contribution of the hot process to the retentions. This kind of analysis is limited to the polymorphic crystals in which structure-sensitive reactions are concerned at the present stage. Although this method is not very accurate (small corrective terms will be discussed elsewhere), it makes it possible to estimate thermal and hot processes which in solids it was hitherto difficult to discriminate between.

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Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid

Several papers dealing with electron spin resonance investigations of irradiated deoxyribonucleic acid (DNA) and its constituents have appeared in the literature[1–5]. In all cases, however, unoriented powder samples have been used. In this communication we wish to report some preliminary findings made with an oriented sample of calf-thymus DNA.

Fibres of the DNA were drawn under controlled humidity conditions (66 per cent relative humidity) and wound on to a 'Spectrosil' grade quartz plate which could be mounted inside a tube[6] of the same material, enabling the fibres to

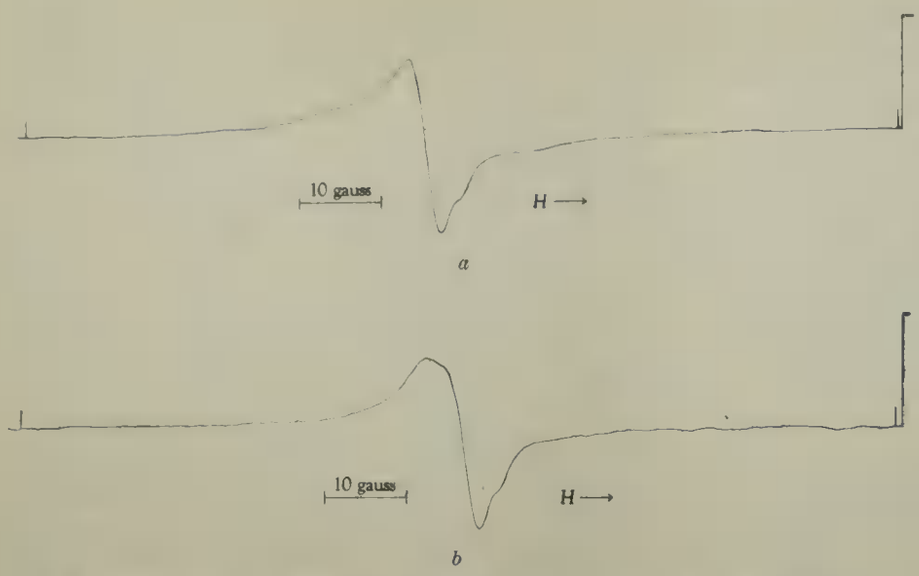

Fig. 1. Electron spin resonance spectra of dry calf thymus DNA fibres X-irradiated at room temperature. *a*, Fibres parallel to the magnetic field; *b*, fibres normal to the magnetic field

be irradiated in an inert atmosphere of known humidity and afterwards transferred to the electron spin resonance spectrometer. The latter was of conventional design, using 100 kc/s modulation and first derivative presentation.

Fig. 1 shows spectra obtained after irradiating the fibres (weight 10 mg) at 0 per cent relative humidity under nitrogen with 250 kV X-rays to a dose of about 1 Mrad at room temperature. Spectra are shown with the magnetic field both parallel and normal to the fibres. The spectrum for the parallel position is approximately symmetric (line width between points of maximum slope $\simeq 4$ gauss), while that for the normal position is somewhat broader and asymmetric. Spectra were recorded over the dose range 0–1·5 Mrad, but were in all cases similar to those of Fig. 1. After the maximum dose (1·5 Mrad) had been given, the radical concentration was estimated to be $5{\cdot}6 \times 10^{18}$ free spins g^{-1} corresponding to a yield $G \simeq 6$ (radicals/100 eV). The radicals showed little evidence of decay over several weeks, the fibres being stored under dry nitrogen at room temperature.

It will be noticed that the spectra are very different from those obtained by Ehrenberg *et al.*[3] for γ-irradiated calf-thymus DNA in two respects. First, our spectra show only a faint trace of hyperfine structure and, secondly, the line width, measured between points of maximum slope, is much less than those reported in the literature. However, when an amorphous sample of the same batch of DNA was irradiated under similar conditions, a spectrum[7] showing a narrow central line (maximum slope width $\simeq 6$ gauss) and well-resolved hyperfine lines was obtained.

An attempt was made to anneal the radicals by immersing the quartz sample tube in warm water for 10 min at successively higher temperatures. Between 60° and 70° C a 50 per cent decrease in radical concentration occurred, while at 80° C there was evidence of the appearance of hyperfine lines, but these were always of low intensity.

When the tube containing the (dry) DNA fibres was opened to the atmosphere, no change in signal was observed after 30 min. The signal disappeared, however, after leaving the fibres exposed to the atmosphere overnight. It has previously been reported that the decay of radiation-produced electron spin resonance signals in DNA appears to depend on the water content[3].

Since DNA is believed to have a helical structure, we consider the expected angular variation of the spectrum of a system of helically distributed radicals. Assuming that the free radical sites have the same orientation as in the undamaged helix, the *g*-value parallel to the helix axis will be the same for each member of a population of repeated radical species irrespective of the helical rotation operation. With the field normal to the helix one has, in general, a distribution of *g*-values depending on the magnitudes and inclinations to the helix axis of the principal *g*-values of the radical species. If the *g*-tensor has cylindrical symmetry, a spread of *g*-values is, of course, obtained only if the unique direction does not coincide with the helix axis. Further consideration of the perpendicular field case shows that, although the spread of *g*-values produces an overall broadening effect, this alone will not give an asymmetric spectrum provided that the fractional change in *g*-value is very small.

Similar considerations apply to the hyperfine coupling tensors in that single values of splittings occur along the helix axis. The parallel field case is analogous to that found in single crystals when site-splittings are absent. Averaging of the spectrum occurs in the perpendicular case when one or more of the tensors exhibits anisotropy in this direction, but the spectrum becomes asymmetric only when hyperfine anisotropy is accompanied by *g*-value anisotropy. It can be shown, for example, that if only an anisotropic doublet is present, the maximum absorption of the smeared-out spectrum is displaced towards the low-field side if the direction of the smaller *g*-value in the perpendicular plane coincides with that of the larger hyperfine splitting.

The foregoing considerations are independent of the exact nature of the radical sites. We can now consider what types of radical are present. As mentioned previously, the spectrum for the parallel orientation shows only slight resolution of components even though the best resolution is expected for this direction. One factor to be considered is the degree of orientation of the radicals within the helices and of the helices within the fibres themselves. If only one type of radical were present, the lack of resolution could be accounted for by poor orientation. However, we should then expect a distribution of *g*-values leading to an asymmetric spectrum. The observed spectrum is nearly symmetric. If we suppose that several radical species are present, the overlapping hyperfine structure characteristic of each producing a poorly-resolved resultant, the *g*-values must lie within narrow limits to account for the relative lack of asymmetry. This would be the case if the radicals were π-electron systems located on the bases.

Now polarized infra-red investigations[9] have shown that, at high humidities at least, the base planes in DNA are nearly normal to the helix axis. Theoretical considerations[10] show that the *g*-values of such radicals normal to the plane of the π-electron system should be nearly equal to the free spin value, with slightly larger in-plane values. Reported experimental data[11] confirm this. The two in-plane values are usually unequal. On this picture we expect all the π-electron radicals to contribute nearly the same *g*-value along the helix axis so that a nearly symmetric spectrum results. At low water contents the bases are tilted off the helix axis; but even if this were by as much as 30° the *g*-value normal to the base planes would still make the largest contribution along the helix axis.

If this picture is correct the asymmetry of the perpendicular spectrum presumably arises both from asymmetry in contributions from any one radical species due to simultaneous *g*-value and hyperfine anisotropy as discussed above, and asymmetry arising from differences in the mean in-plane *g*-values of the various species supposed to be present. In practice, we cannot distinguish these two effects.

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BIOPHYSICS

Computer Recognition of Cervical Cell Nuclei

Computer programmes which process data obtained by scanning 35-mm films of individual cervical cells are being tested in an attempt to obtain a quantitative definition of the differences between normal and malignant cells. The scanning instrument is of an electro-optical nature and has a digital output[1,2]. It scans the photographic film in an array of points with each point yielding a number (between 0 and 128) the value of which depends on the emulsion density. The scan resolution can be varied from 4 lines each with 4 points to 256 lines each with 512 points. A typical single line scan (with 128 points) across a cervical cell nucleus is seen in Fig. 1. Normally these data are punched out automatically on paper tape, suitable for input to a Ferranti *Sirius* computer.

Having transformed the cell into an array of numbers, the problem now is to extract from it parameters which show significant differences between normal and malignant cells. It is essential for the solution of this problem that the original geometry of the cell nuclei be preserved. This has now been achieved in spite of the difficulties encountered when a cytoplasm is present.

The successful preservation of the geometry is illustrated by the graphs in Figs. 2 and 3, which were obtained after the computer had processed data obtained from scanning a normal cell and a 'malignant' cell. The object was to obtain a measure of the apparent thickness of the nuclear surface membrane. As a check on the validity of the computer programme the same sets of data were processed manually. These results are plotted beside the computer's selections.

The results for the nuclear surface membrane are taken from only a small part of the computer programme which is designed to extract a number of parameters characteristic of the cell. It was because of the ease of visual display of the nuclear surface membrane that it was selected for presentation here.

At the moment all these parameters are being examined with the view of obtaining quantitative definitions of the various classifications of cervical cells.

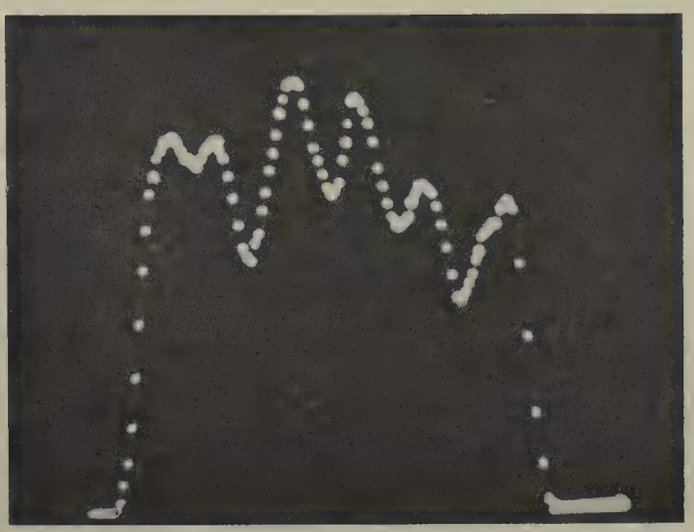

Fig. 1

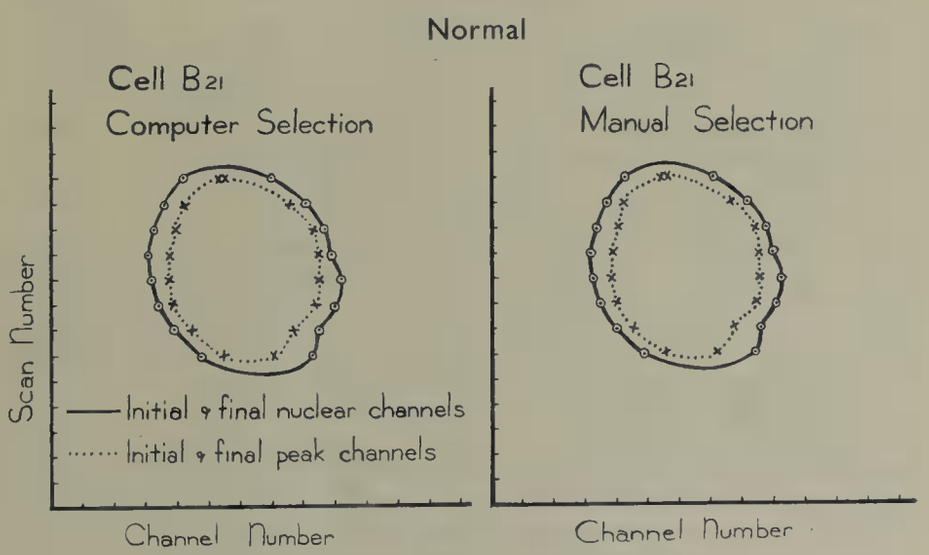

Fig. 2

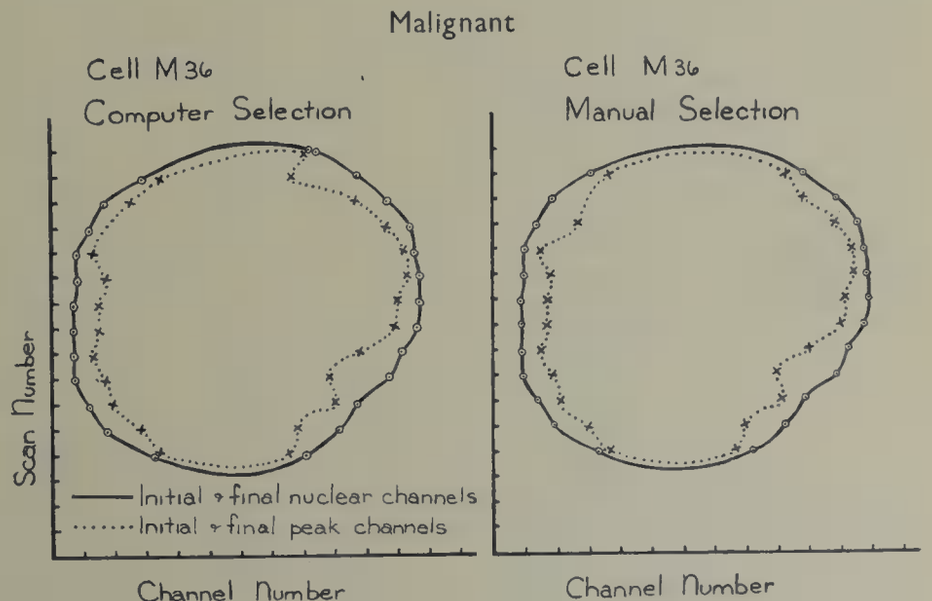

Fig. 3

I thank Dr. A. Ward for his advice and Mr. John Gray for assistance.

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University of Strathclyde.

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Distribution of Deoxyribonucleic Acid along Human Chromosomes

To the physicist the structure of the 46 normal human chromosomes is a problem analogous to that of atomic structure with as much fundamental significance. Although the appearance of artefacts and deviations resulting from variations in the technique of preparing samples of human chromosomes introduces, at this time, a degree of error into the results, it is possible to consider the measurement of the distribution of deoxyribonucleic acid (DNA) along the chromosome as essentially a purely physical problem of structure.

Elsewhere the electronic instrumentation involved in this work has been described in some detail[1–4]. Briefly, the equipment consists essentially of a flying-spot scanner which scans a 35-mm transparency of (in this case) a human chromosome and converts the image into an array of numbers representing the grain densities involved. In the work described here the scan consists of 16 lines each containing 32 points and the grain density on any one point is represented by an integral number lying anywhere between 0 and 128. That is to say, the image of the human chromosome is scanned with 128 shades of grey.

The decision to limit the scan to a matrix of 16 × 32 was made for technical reasons only, and it is hoped later to increase this matrix as the equipment is improved. Nevertheless, even at present, using a 16 × 32 matrix and bearing in mind the limitations in the image due to diffraction and artefacts, the present matrix appears to be of considerable use in discovering the DNA distribution along the chromosomes. It is anticipated that, as the techniques of preparing the human chromosomes for this work steadily improve and artefacts are reduced and as the measuring equipment improves, it will be possible to make a more and more carefully detailed study of the DNA distribution, that is to say the physical structure of the human chromosome. The results described here may be regarded as a preliminary survey indicating the likely final development of the technique. Eventually, when the structure of the normal human chromosomes has been discovered in sufficient detail, the technique will be applied to a study of human chromosomes associated with different physiological conditions in human beings, particularly those involving malignant growths.

For convenience, chromosomes 4 and 12 were selected for study, as they are obviously different when viewed by the human eye. The transparencies of the chromosomes were inserted into the scanning instrument and the images of each of the chromosomes were transformed into a 16 × 32 matrix of numbers. These numbers were observed visually on a display tube as a check on the scanning procedure and then punched out on paper-tape. The paper-tapes were analysed by a Ferranti *Sirius* computer programmed to provide two forms of output data. The first form consists of a geometrical print-out, an array of crosses indicating when a number was above an arbitrarily selected background-level. The result of such a print-out is shown in Fig. 1 for chromosomes 4 and 12. The second form in which the data appear from the *Sirius* computer consists of a series of 32 numbers; each of these numbers represents the sum total of all the numbers appearing in that particular vertical column of the original matrix. That is to say, these numbers represent the total DNA at a particular point along the chromosome, the distance along the chromosome being determined by which of the particular columns is being considered. This type of analysis is particularly interesting, as a graph of these 32 numbers gives essentially the DNA distribution along the chromosome, and typical results for chromosomes 4 and 12 are shown in Fig. 2.

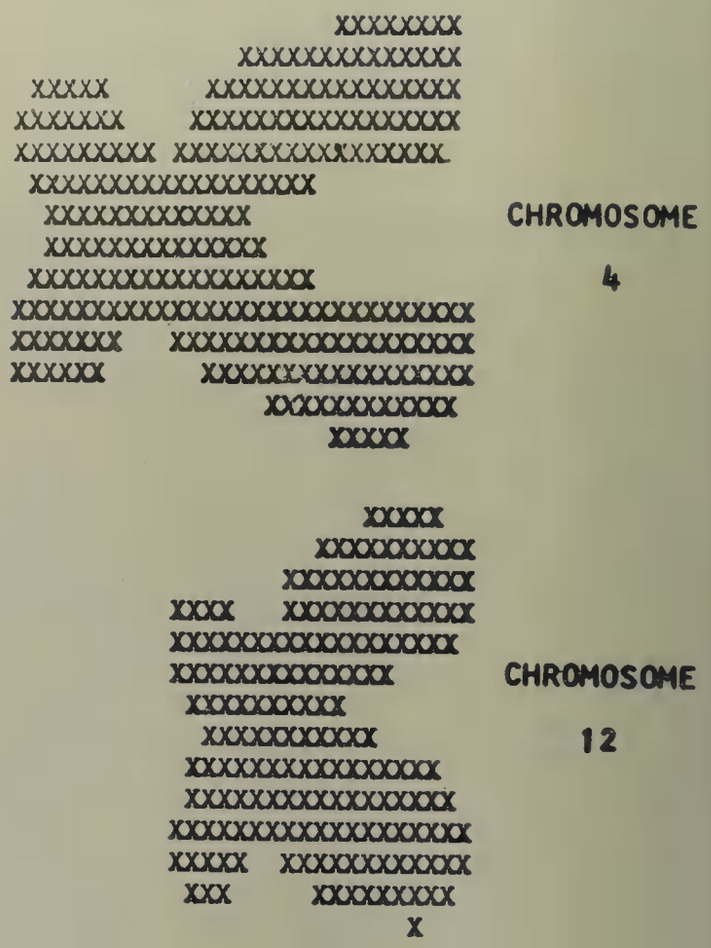

Fig. 1

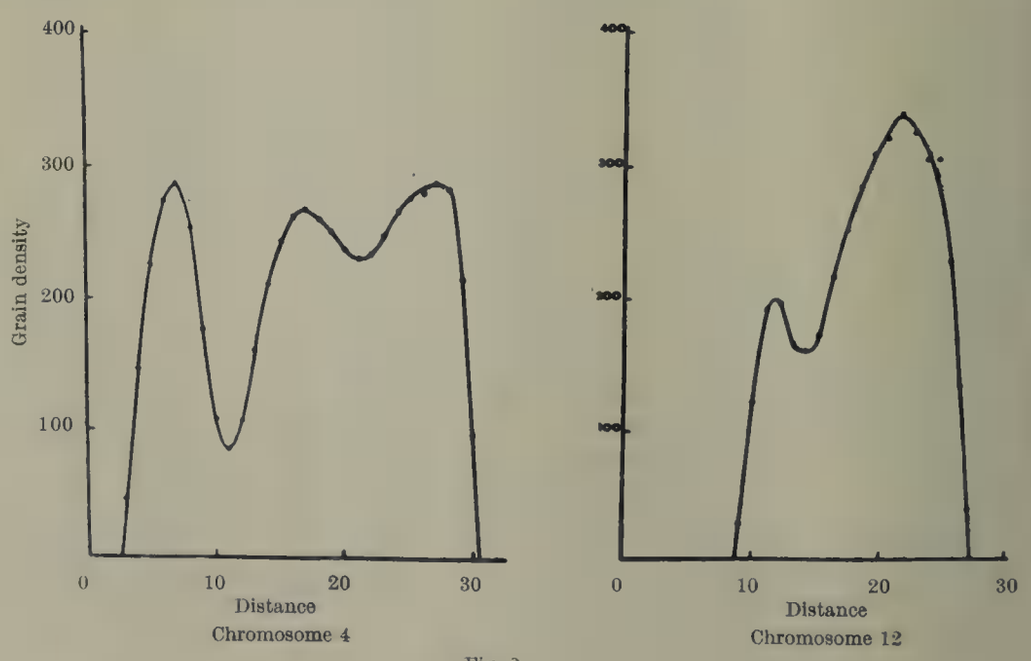

Fig. 2

It can be seen from Fig. 2 that the variation in DNA distribution along the two chromosomes is quite striking although it should be stated that these are preliminary results and there is some reason to believe that the stain being used may in itself tend to be taken up with variable efficiency along the chromosome and therefore the stain density, which is of course the quantity being measured, is not a very accurate representation of the DNA distribution. This factor of the best choice of stain is being investigated at this laboratory, and it is hoped to find a stain which is sufficiently dark for scanning purposes and which also is taken up uniformly by the DNA present.

Other results on chromosomes 4 and 12 show essentially the same type of structure as indicated in Fig. 2, and a programme is now under way to discover the essential features of the DNA distribution along all the 46 normal human chromosomes.

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BIOCHEMISTRY

Secretion of Newly Synthesized Insulin *in vitro*

Up to the present, there has been little information available concerning the rate at which newly synthesized protein hormones may be secreted, following the stimulation of endocrine glands. In recent work on the synthesis of insulin within pancreas slices, *in vitro*, it became of interest to determine whether or not newly synthesized insulin may be released directly into the incubation medium, or alternatively whether or not release of insulin might be delayed following its accumulation within β-granules. It has already been suggested by Lacy[1] that release of insulin from β-cells in response to glucose involves the movement of these granules to the cell surface, where they are released into blood vessels.

In the experiments to be reported, insulin was labelled by incorporation of label into pancreas slices *in vitro*, and its release into media containing glucose studied. When rabbit pancreas slices are incubated in physiological media, such as Krebs–Ringer bicarbonate, there is a small release of insulin at low glucose concentrations, though this is very greatly increased when the sugar concentration is raised. The increased release of insulin has been shown to reflect, at least qualitatively, the physiological behaviour of β-cells *in vivo*[2]. To label the insulin, rabbit pancreas slices were incubated for 1 h with tritiated leucine of high specific activity by methods which have already been described in detail elsewhere[3,4]. The incubation medium was then poured off and the pancreas slices washed thoroughly with fresh medium to remove excess radioactivity. The slices were then incubated for 30 min in a medium containing low concentrations of glucose (50 mg/100 ml.). For the final 1-h incubation phase, the medium was again discarded and the pancreas slices divided into two parts, one being incubated in a medium of low glucose concentration (50 mg/100 ml.), the other being incubated in medium containing a much higher concentration of glucose (250 mg/100 ml.). The medium was gassed with 95 per cent oxygen and 5 per cent carbon dioxide before each incubation phase. Insulin in the medium derived from the final incubation phase was assayed by an immunological method[5], and isolated from the medium so that its specific radioactivity might be determined.

The isolation of labelled insulin in the medium was carried out by an immunological technique, based on the precipitation of an insulin–antibody complex by means of an anti-γ-globulin. For this purpose, the whole medium was dialysed against distilled water and freeze dried. The proteins were then dissolved in a small volume of 0·003 M hydrochloric acid, and allowed to react with 0·5 ml. of a potent anti-serum made by repeated injection of crystalline ox insulin into guinea-pigs[6]. The whole γ-globulin fraction was then precipitated by the addition of a rabbit–anti-guinea-pig γ-globulin serum. The precipitate obtained was spun off, and after washing with saline, the insulin was regenerated from it by acid, and further purified by two-dimensional chromatography and electrophoresis on paper as previously described[4]. Insulin regenerated was assayed in the extract by immunoassay. The results of two representative incubations are shown in Table 1.

Table 1

Exp.	Insulin release into medium in final incubation phase (mU/flask/h)		Specific radioactivity of insulin extracted from medium (c.p.m./μg)	
	Low glucose	High glucose	Low glucose	High glucose
1	6·48	24·4	1,040	567
2	7·36	26·1	912	552

It will be seen that when pancreas slices are incubated in a medium containing a low concentration of glucose for 1·5 to 2·5 h after the start of labelling, there is some release of labelled insulin. However, when the final phase of incubation is in a medium of high glucose concentration, insulin release from the slices is increased several-fold while the specific activity of the insulin falls markedly. Very recently, similar results have been obtained from experiments in which the medium was extracted with acidic ethanol and the crude insulin precipitated with ether-ethanol[7] before purification by the method already described here. In two such experiments the ratio of insulin output in the high glucose medium to that in medium of low glucose content was 4·08 : 1, while the ratio of specific activities of the insulin extracted was 0·35 : 1. It seems, therefore, that the greater part of the insulin secreted in response to a glucose stimulus has not been synthesized in the preceding 1·5–2·5 h. These results are in marked contrast with those obtained for the secretion from liver of newly synthesized albumin which is known not to be stored in granules[8].

In pancreatic acinar tissue there is evidence from autoradiographic studies that newly synthesized secretory proteins appear first in the rough surfaced elements of the endoplasmic reticulum, then in the Golgi complex and finally after 1 h in the zymogen granules, from which they begin to be discharged 2 h after synthesis[9]. From the results described here, it seems possible there may be analogies between the secretion of newly synthesized insulin and the discharge of recently made secretory proteins.

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Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius

In perfused rat heart and isolated rat diaphragm the rate of release and oxidation of fatty acids from muscle glycerides is increased by alloxan-diabetes[1]. These changes in diabetes involve actions of growth hormone and corticosteroids in insulin-deficient rats because they are not seen in muscles of hypophysectomized diabetic rats unless the animals are treated with growth hormone and cortisol[1]. As part of an investigation of the mechanisms of these changes we have investigated the effects of diabetes, hypophysectomy and treatment with growth hormone and cortisol on the concentrations of glycerides and phospholipids in rat heart and gastrocnemius (the latter as a representative skeletal muscle). The methods and procedure were as follows.

Rat hearts were perfused for 10 min with medium containing glucose (5·5 mM) and insulin (0·1 unit/ml.), and the excised ventricles frozen with a tissue clamp and powdered in a percussion mortar[2]. Gastrocnemius muscle was frozen in acetone–solid carbon dioxide mixture and powdered after 10 min of hind-limb perfusion through the abdominal aorta with the foregoing medium containing glucose (11 mM) but no insulin. These perfusions remove blood and blood lipids. Details of induction of alloxan-diabetes and treatment with growth hormone and cortisol were as described elsewhere[2]. The frozen muscle powder was extracted with chloroform/methanol (2 : 1 v/v) (15–30 ml./g) in a Potter homogenizer, allowed to stand at 0° for 2 h and centrifuged. The supernatant was shaken with 0·3 vol. of 4 mM magnesium chloride and phases separated by centrifugation after 30 min. The chloroform phase was evaporated to dryness at 70° and the residue taken up in about 7 ml. of chloroform and a sample (0·5 ml.) taken for determination of phospholipid-P (ref. 3), after evaporation of chloroform and oxidation with 0·15 ml. concentrated $H_2SO_4/HClO_4$ (2 : 1 v/v). The remainder was shaken with 0·5 g silicic acid to remove phospholipid[4] (confirmed by assay of phospholipid-P). A sample of supernatant (4 ml.) was evaporated to dryness and glycerides separated by thin-layer chromatography on 'Silica Gel *G*' (Merck) using as solvent petroleum ether (fraction 40°–60°)/di-ethyl ether/methanol/acetic acid (90 : 20 : 2 : 3 v/v). As markers crude triolein (British Drug Houses, Ltd.) containing mono- and di-olein, and pure tripalmitin and mono-olein (Hormel Institute, University of Minnesota), were used (about 0·5 μmole). The R_F values of the separated classes were: monoglycerides 0·05; diglycerides 0·45; triglycerides 0·90. The spots, located by markers, were scraped from the plate and extracted twice with 2 ml. of chloroform/methanol (2 : 1 v/v). The extract was evaporated to dryness at 70°, saponified with 0·5 ml. 4 per cent potassium hydroxide in 95 per cent ethanol for 30 min at 60°, acidified with 0·5 ml. 10 per cent $HClO_4$, neutralized with saturated $KHCO_3$, and $KClO_4$ separated by centrifugation at 0°. Glycerol was assayed enzymatically in the supernatant[5]. Recoveries (using 1-[^{14}C]-palmitate labelled tripalmitin from the Radiochemical Centre, Amersham, added to extracts of heart) were in excess of 90 per cent. Recovery of mono-olein was in excess of 80 per cent. Contamination with phospholipid-P was less than 0·05 per cent.

The results are shown in Table 1. In the glyceride series the concentration of triglyceride was greatly in excess of that of di- and mono-glyceride, the concentration ratios being 140 : 1 : 6 in normal rat heart and 120 : 1 : 4 in normal rat gastrocnemius. These values indicate that loss of the first fatty acid may limit triglyceride breakdown in muscle. The concentration of triglyceride in heart and gastrocnemius was significantly increased by alloxan-diabetes. In hypophysectomized rats alloxan-diabetes failed to increase the triglyceride concentration unless the animals were treated with growth hormone and cortisol. The concentration of diglyceride (measured on pooled extracts) may also have been increased in alloxan-diabetes whereas the concentrations of monoglyceride and phospholipids appeared to be unchanged. When hearts from normal rats were perfused for 60 min without substrate the triglyceride concentration fell from 18·7 ± 0·8 (8) to 8·7 ± 0·7 (8) (μmoles/g dry wt.; mean ± *S.E.* with number of hearts). No significant change in phospholipid was detected (186 ± 6–171 ± 7).

Table 1. Glyceride and Phospholipid Concentrations in Rat Heart and Gastrocnemius

Rat	Tissue	Concentration μmoles/g dry wt. (Mean ± *S.E.*) Triglyceride	Diglyceride	Monoglyceride	Phospholipid phosphorus
Normal (6)	Heart	13·6 ± 1·4	0·1	0·6	184 ± 8
Alloxan-diabetic (6)		* 28·2 ± 1·9	0·6	0·7	163 ± 8
Hypophysectomized (6)		10·3 ± 1·3	0·4	0·7	143 ± 4
Hypophysectomized diabetic (6)		10·6 ± 0·9	0·3	0·7	154 ± 4
Hypophysectomized diabetic treated growth hormone and cortisol (4)		* 22·9 ± 3·7	–	–	142 ± 5
Normal (8)	Gastrocnemius	12 ± 1·5	0·1	0·4	64 ± 2
Alloxan-diabetic (8)		26 ± 2·7	0·4	0·5	63 ± 1

*$P < 0·01$ versus control. No. of muscle samples given in parentheses.

These observations lead us to conclude that heart muscle triglyceride is broken down during perfusion, thus providing fatty acid for oxidation[6]. Evidence for the location of this glyceride in muscle cells has been presented[1,7]. The concentration of triglyceride, but not that of phospholipid, is increased by actions of growth hormone and cortisol in an insulin-deficient rat. Loss of the first fatty acid from triglyceride appears to limit breakdown in both normal and diabetic rats. The role of increased triglyceride concentration in accelerated lipolysis in diabetic muscle[1] and the source of muscle glyceride (that is, whether derived from plasma glyceride or plasma not-esterified fatty acids) is under investigation.

We thank Mr. R. O. Law for help with perfusion of hind limbs and the British Diabetic Association, the Medical Research Council and the Royal Society for grants.

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Acrylamide-gel Electrophoresis of β-Lactoglobulins stored in Solutions at *p*H 8·7

Native β-lactoglobulins-*A* and -*B*, mol. wt. 35,500, can reversibly dissociate into sub-units of mol. wt. 17,750 in acidic[1] or alkaline[2] solutions. Aggregation of β-lactoglobulin-*A* to a tetramer structure has been observed[1] in solutions of *p*H 3·5–5·2 stored at 4° C. Irreversible denaturation of β-lactoglobulin occurs on heating[3] neutral solutions above 65° C or on storage[4,5] in alkaline solution at 25° (slowly in the *p*H range 8–9·5, more rapidly in solutions of higher *p*H). This denaturation was shown by changes in iso-electric solubility, optical rotation and light scattering; Tiselius electrophoresis in 'Veronal' (*p*H 8·4) buffer separated one, or occasionally two, faster-migrating components in addition to native β-lactoglobulin. An initial reversible splitting followed by irreversible aggregation was postulated[3], but the nature and number of products formed were not studied further. Bingham, Krugman and Estermann[6] recently noted that stored samples of β-lactoglobulin were separated by acrylamide disc electrophoresis[7] into five bands whereas the fresh material split into only two bands; they gave no indication

of the *p*H or composition of the β-lactoglobulin samples. This communication reports the progressive formation of at least eleven new species when β-lactoglobulin-*A* or -*B* or -*A*/*B* is stored in alkaline solution.

Twice-crystallized β-lactoglobulin-*A* and -*B* genetic variants were separately prepared[8] from the milks of individually-typed Ayrshire cows[9], and the *A*/*B* mixture from pooled herd milk, and each was freeze-dried. Each was electrophoretically pure (Fig. 1, *a*, *b*, *c*). The protein (10 mg) was dissolved in buffer solution (1 ml.) consisting of 0·076 M *tris* titrated to *p*H 8·7 with HCl and containing 0·10 M NaCl, and stored for several days at 2° C. Aliquots were removed daily for electrophoretic examination.

Sample solutions containing 100 μg of β-lactoglobulin were separated by vertical disc electrophoresis[7] in 11 per cent acrylamide-gel rods. The gels were prepared in 63 × 5 mm glass tubes using 'Cyanogum 41' (a mixture of acrylamide and methylene*bis*acrylamide; B.D.H., Ltd., Poole, England) dissolved in 0·076 M *tris* titrated to *p*H 8·6 with citric acid. The electrode tanks contained 0·3 M boric acid titrated to *p*H 8·5 with sodium hydroxide[10]. It was not necessary to use the system[7] of additional 'spacer' and 'sample' gels of weaker acrylamide concentration polymerized on top of the main gel column; the sample solution, made more dense by addition of 10 per cent sucrose and made visible by a trace of bromophenol blue, was layered directly on to the flat top of the 11 per cent acrylamide gel underneath the tank buffer solution. After electrophoresis for about 1 h at a constant current of 4 m.amp per gel rod, the rods were stained with amido black and electrolytically de-stained. The gel rods were stored in 7 per cent acetic acid, and photographed under strong back-lighting.

The electrophoretic patterns revealed a progressive formation of slower-migrating protein bands as the storage period increased. After one day about three new bands were visible (Fig. 1, *d*, *e*, *f*) and after seven days there were at least eleven sharp, slower-migrating bands. The β-*A* and β-*B* and β *A*/*B* mixture appeared to behave similarly, giving equal numbers of new bands at the same rate. After seven days storage nearly all the original β-lactoglobulin bands had disappeared (Fig. 1, *g*, *h*, *i*) from the alkaline solution, but a control sample of 1 per cent β-lactoglobulin in 0·076 M *tris* titrated to *p*H 7·0 with hydrochloric acid still showed only the original (fast) β-lactoglobulin band and no new bands. Portions of 7-day-old alkaline solutions were titrated to *p*H 6·0 and examined after storage at this *p*H for several days further, but their electrophoretic patterns remained identical to those of the 7-day-old alkaline solution. This shows that the formation of the new species is induced by the high *p*H and is not reversible by subsequent acidification. Because of the molecular-sieving effect of 11 per cent acrylamide gels[11], it is probable that the slower-migrating bands represent progressively higher molecular weight aggregates formed slowly from either the original β-lactoglobulin molecules (mol. wt. 35,500) or their half-units. The amount of aggregation did not cause precipitation from the *p*H 8·7 solutions even after storage for several weeks, and no protein material remained on the origin of the gel rod after electrophoresis.

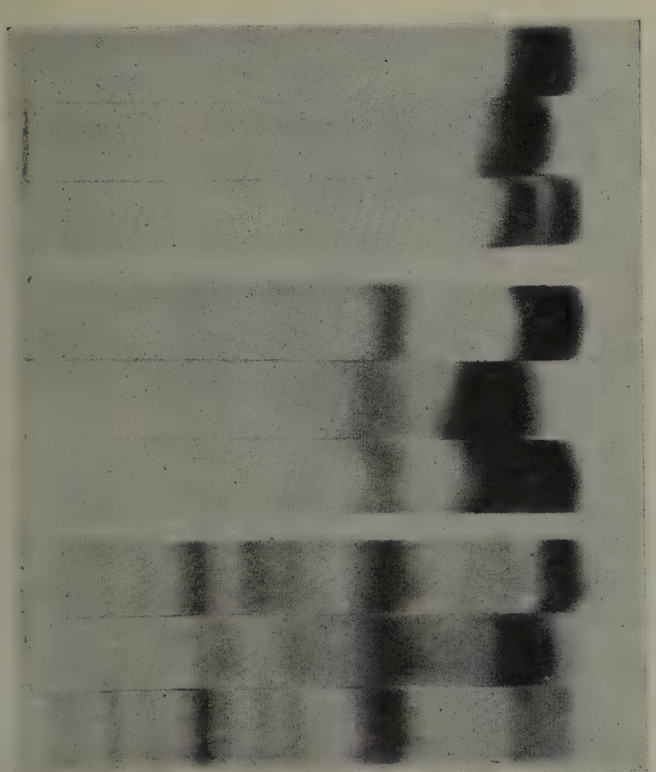

Fig. 1. Patterns showing progressive aggregation of a 1 per cent β-lactoglobulins-*A*, -*B* or -*A*/*B* mixture on storage in *tris* buffer (*p*H 8·7) at 2° C. Disc electrophoresis was performed in 11 per cent acrylamide gel rods (details given in text). *a*, β-*A* solution, fresh made; *b*, β-*B* solution, fresh made; *c*, β-*A*/*B* solution, fresh made; *d*, β-*A* solution, 1 day old; *e*, β-*B* solution, 1 day old; *f*, β-*A*/*B* solution, 1 day old; *g*, β-*A* solution, 7 days old; *h*, β-*B* solution, 7 days old; *i*, β-*A*/*B* solution, 7 days old. The sample application point is at the left-hand side of the photographs

Probably the new species result from alkaline oxidation of cysteine residues, linking adjacent β-lactoglobulin molecules by a disulphide bond. The similarity of heat- and alkali-induced aggregation is now being investigated. The foregoing results emphasize that β-lactoglobulin or whey protein solutions should always be freshly prepared for routine electrophoretic examination and not stored in alkaline solution.

I thank Mr. M. Rogers for his help.

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Haemoglobin Polymorphism in the Cod (*Gadus morrhua*): a Single Peptide Difference

Haemoglobin of the cod (*Gadus morrhua*), when submitted to agar-gel electrophoresis at *p*H 7·3, shows a clear polymorphism.

Using this technique Sick[1] showed the existence of three patterns (Fig. 1) suggesting a single gene control for the fast bands Hb I1 and Hb I2. Furthermore, he found the

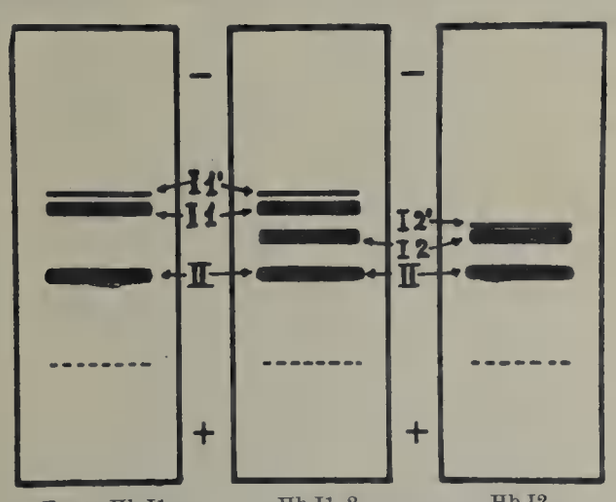

Fig. 1. Tracings of patterns of cod haemoglobin subjected to agar-gel electrophoresis, phosphate buffer 0·02 M, *p*H 7·3

frequencies of the three types, I1, I2, I1–2, to be in good agreement with the expected values according to the Hardy–Weinberg law.

In order to investigate the nature of this polymorphism in terms of structural differences, we analysed several samples of cod haemoglobin by means of the 'fingerprinting' technique.

Whole blood samples, collected by heart puncture in ACD solution, were washed and haemolysed, and the agar-gel electrophoresis was performed at once, following the technique of Sick. The three patterns were easily found.

The haemoglobins of the same type were pooled together, and purified by starch-block electrophoresis ('Veronal', 0·05 M; *p*H 8·6). Since the two bands Hb I1 and Hb I2 of the heterozygotes are not resolved with this method, the fast bands I1 and I2 of the homozygotes were isolated, checking the purity of the eluted fractions by agar-gel electrophoresis. From the eluted haemoglobin, globin was prepared (by the method described in ref. 2, modified by using low temperature). The globin was then submitted to tryptic hydrolysis, using a *p*H-stat, and fingerprinted[3].

The patterns obtained (Fig. 2) were absolutely identical for the two types examined. The specific staining for arginine, tyrosine, histidine, tryptophan and *S*-amino-acids also failed to reveal any differences.

The use of a different solvent for the chromatography[4], the analysis of the neutral zone of the fingerprint by high-voltage paper electrophoresis at *p*H 3·5, and the 'extended fingerprint' technique[5] also gave the same negative result.

The globin was then submitted to chymotryptic digestion, and fingerprinted[6]. The patterns of the two types I1 and I2 were found to be exactly the same, except for a clear peptide spot in Hb I1 with no counterpart in Hb I2 (Fig. 3). The extra spot was also present, more faintly coloured with ninhydrin, in Hb I1–2 (the heterozygote type in Sick's hypothesis). The specific staining showed the extra peptide to contain hystidine, but no other differences were found on comparing the patterns of the two types. Again, using the solvent suggested by Baglioni, the analysis of the neutral zone peptides by paper electrophoresis at *p*H 3·5, and the extended fingerprint revealed no other difference than the extra peptide in Hb I1.

The trypsin-resistant 'core' was then analysed by digestion with chymotrypsin and fingerprinting. The 'core' was

Fig. 2. Tracing of a typical fingerprint of trypsin-digested cod haemoglobin. The pattern is the same for type Hb I1 and type Hb I2. A, Ty, Tr, H and S denote positive reaction to the staining for arginine, tyrosine, tryptophan, histidine and sulphur-containing amino-acids

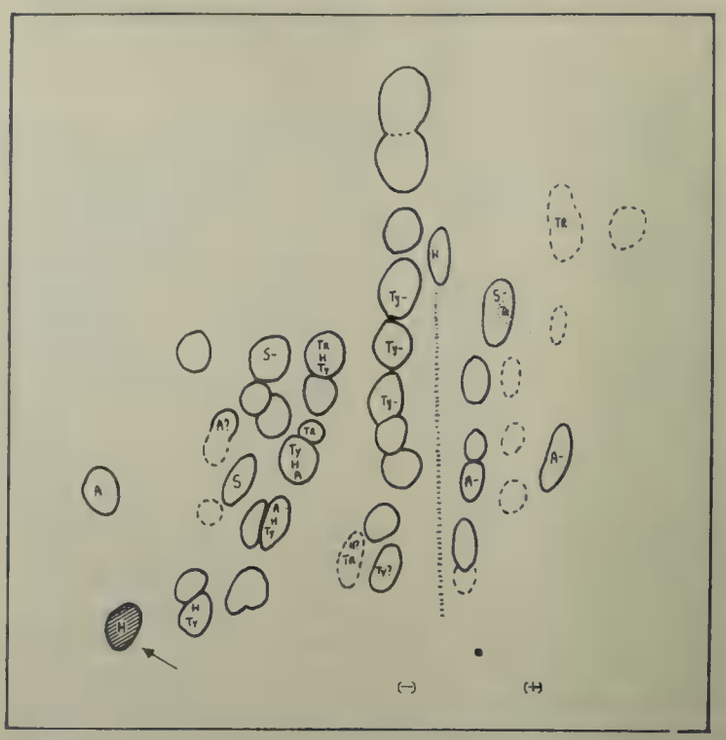

Fig. 3. Tracing of a typical fingerprint of chymotrypsin-digested cod haemoglobin. The shadowed spot is present in type Hb I1 only

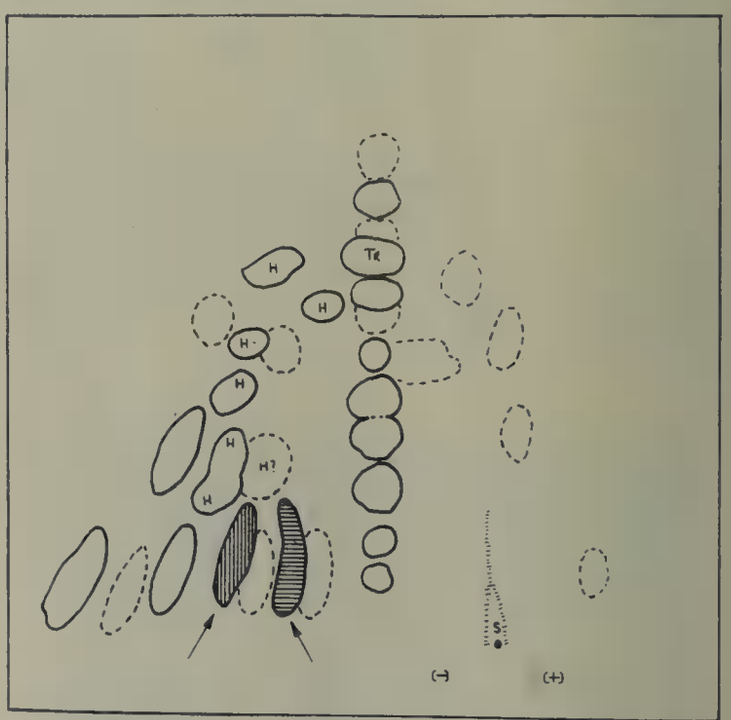

Fig. 4. Tracing of a typical fingerprint of the trypsin-resistant 'core' digested with chymotrypsin. The horizontally shadowed spot (right) is present in type Hb I1; the vertically shadowed spot (left) is present in type Hb I2

obtained by spinning down at *p*H 8 the undigested globin of a tryptic digest, lowering the *p*H of the supernatant to 6·5, and heating it at 100°; the precipitated peptides were then spun down, and washed three times with diluted Michl's buffer, *p*H 6·4.

The 'cores' of the two types I1 and I2, digested with chymotrypsin, gave fingerprints differing only by the position of one peptide, moving a little faster towards the cathode in type I2 (Fig. 4).

The specific staining and the alternative methods described did not reveal any other differences between the two patterns. It should be mentioned that the critical peptides did not stain for histidine.

Two questions are raised by these findings, namely: (1) What is the difference, in terms of amino-acid composition, between the two peptides with different mobility in the fingerprints of the chymotryptic-digested 'cores'? Since

the change in position is indeed small, the substitution, if any, should involve two neutral amino-acids. A preliminary analysis seems to suggest that this is so. (2) What is the relationship between the aforementioned 'core' peptides and the 'extra' one in Hb I1 chymotryptic digest? The answers will come only from sequence analysis of the three peptides.

This work was carried out in the Laboratory of the Department of Paediatrics of the University of Groningen. We thank Prof. J. P. H. Jonxis for his help and advice, and Miss du Vigny, Royal Institute for Fishery Research, Ijmuiden, Holland, for the blood samples.

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Factors affecting the Amount and Composition of the Serum Seromucoid Fraction

The glycoprotein-rich seromucoid fraction[1] is isolated by first precipitating most serum proteins with perchloric acid—seromucoid being precipitated from the filtrate with phosphotungstic acid. The solubility of certain glycoproteins in perchloric acid is assumed due to covalently bound hexose, hexosamine, fucose and sialic acid residues. Thus, seromucoid contains perchloric acid-soluble orosomucoid, Zn-α_2-glycoprotein, haemopexin, the haptoglobins and 3·5 *S* α_1-glycoprotein[2] with total carbohydrate and sialic acid contents above 8·0 and 3·3 per cent respectively. Insoluble glycoproteins, absent from seromucoid, include α_2-macroglobulins, ceruloplasmin and transferrin with less carbohydrate content. Seromucoid also contains small amounts of co-precipitated albumin, pre-albumin and γ-globulins which, when pure, are perchloric acid-insoluble.

If a causal relationship were to exist between glycoprotein solubility and carbohydrate content, then removal of carbohydrate residues from glycoproteins should decrease their solubility in perchloric acid and lead to decreased seromucoid precipitation. Since terminally-bound sialic acid residues on glycoproteins can be selectively removed by neuraminidase, the effect of this carbohydrate on glycoprotein solubility in 0·6 M perchloric acid has been investigated.

Normal human serum, containing 60 mg glycoprotein-bound sialic acid/100 ml., was used; the seromucoid-level was 103 and 16·7 mg/100 ml., expressed in terms of protein and hexose contents respectively. Seromucoid was precipitated[3] before and after treatment of serum with neuraminidase (*E.C.* 3.2.1.18), the source of which was receptor destroying enzyme (R.D.E.) from *Vibrio cholerae* (Burroughs Wellcome and Co., London). To 10 ml. of serum were added 10 ml. of 0·2 M acetate buffer (*p*H 5·5, containing 10^{-2} M calcium acetate) and 3 ml. 0·1 N hydrochloric acid, the final *p*H being 5·5. To 4-ml. aliquots were added 2 ml. R.D.E. These mixtures were incubated at 37°. At varying time intervals (0–24 h) 24·8 ml. 0·9 per cent sodium chloride was added to each mixture, and free sialic acid estimated in 0·2 ml. aliquots[4]. To 30 ml. of the remaining mixture were added 14·96 ml. of 1·8 M perchloric acid; the precipitated proteins were separated by filtration through No. 50 Whatman filter paper after 10 min. The seromucoid fraction was then precipitated from 32 ml. of the filtrate with 6·4 ml. of phosphotungstic acid reagent (5 per cent, w/v, in 2 N hydrochloric acid), and measured turbidimetrically at 400 mμ after standing for 15 min. The fraction was then centrifuged down, washed with 10 ml. 95 per cent (v/v) ethanol, dissolved in 4 ml. 0·1 N sodium hydroxide, and estimated for hexose and protein[5]. As is seen in Fig. 1, the enzymatic removal of sialic acid from serum glycoproteins was accompanied by decreased seromucoid precipitation. Sialic acid residues evidently confer hydrophylic properties on some serum glycoproteins, their removal decreasing glycoprotein solubility in perchloric acid, thus decreasing the amount of seromucoid precipitated from the perchloric acid filtrate by phosphotungstic acid. This effect was particularly noticeable when seromucoid was estimated turbidimetrically[6].

Glycoproteins with decreased perchloric acid-solubility following sialic acid removal are probably relatively low in carbohydrate content since purified orosomucoid, containing 40 and 12 per cent total carbohydrate and sialic acid respectively, remained perchloric acid-soluble after the complete removal of sialic acid, presumably because the amounts of hexose and hexosamine remaining were sufficiently high to maintain solubility.

The presence in seromucoid of serum proteins which are normally perchloric acid-insoluble may result from the 'protective' properties of glycoproteins[7]. The protective effect of seromucoid on the precipitation of albumin by perchloric acid was therefore investigated, using seromucoid (containing 4 per cent sialic acid) isolated from normal serum by the precipitation method described in Fig. 1. To five tubes containing albumin (0·8 mg), dissolved in 0·1 ml. 0·9 per cent sodium chloride, were added increasing amounts of seromucoid (0–1 mg), dissolved in 0·1 ml. 0·05 N sodium hydroxide. To each tube was added 3 ml. phosphate buffer (0·2 M, *p*H 7·4) followed by 1·6 ml. 1·8 M perchloric acid; the albumin precipitated was measured turbidimetrically at 400 mμ after 10 min. From Fig. 2, it is evident that seromucoid protected albumin from precipitation by perchloric acid.

Serum glycoproteins function as protective colloids, probably enabling small amounts of normally insoluble substances, such as albumin, pre-albumin and γ-globulins, to remain soluble on the addition to serum of perchloric acid, addition of phosphotungstic acid to perchloric acid filtrates causing them to precipitate with the seromucoid fraction. The presence of these normally insoluble substances in seromucoid, therefore, depends on the amount and protective efficiency of serum glycoproteins, which may vary in pathological conditions.

Factors affecting seromucoid precipitation are of some importance, since these levels vary in disease[8] and are claimed to be of value in cancer diagnosis and in assessing response to treatment[9]. This is particularly relevant in

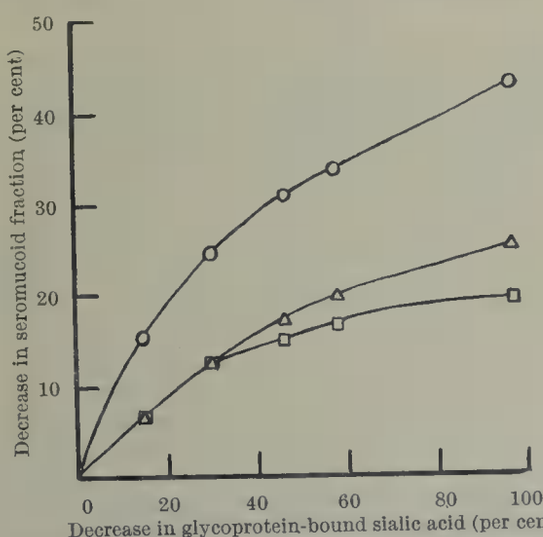

Fig. 1. Decreased precipitation of seromucoid following the removal of sialic acid from serum glycoproteins. Seromucoid-levels were determined turbidimetrically (○), and by estimation of seromucoid protein (△) and hexose (□) contents. Results are expressed as the percentage decrease in the seromucoid fraction following the progressive enzymatic removal of sialic acid from serum glycoproteins

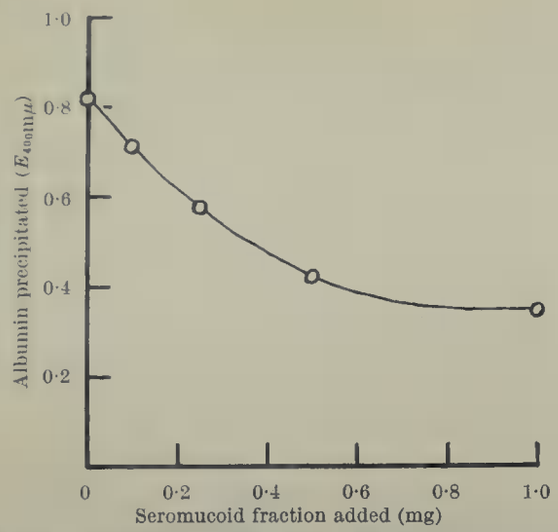

Fig. 2. Protective effect of seromucoid on the precipitation of albumin by 0·6 M perchloric acid. Albumin was estimated turbidimetrically at 400 mμ

view of recent reports that orosomucoid, isolated from the serum of patients with certain diseases, is sialic acid-deficient[10,11]. If other serum glycoproteins also lack sialic acid, low seromucoid-levels may result due to decreased glycoprotein solubility in perchloric acid.

Whether the low seromucoid-levels observed in patients with parenchymatous liver diseases[12] result from defective synthesis in the liver[13] producing sialic acid-deficient, perchloric acid-insoluble glycoproteins must await further knowledge of the composition of these substances in abnormal sera, the perchloric acid-solubility and protective properties of which appear dependent on the nature and amount of covalently bound carbohydrates.

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PHYSIOLOGY

Tubular Site of Urate Secretion in the Rabbit

In the rabbit a pronounced secretion of urate during infusion of urate has been shown[1]. In the Dalmatian dog secretion of urate may be readily demonstrated, while re-absorption of urate prevails in the mongrel (that is, non-Dalmatian) dog. A proximal re-absorption of urate in the mongrel dog has consistently been observed by the stop-flow method[2–4], whereas secretion has been found at this site in the Dalmatian dog[2,4]. Yü *et al.*[4] have suggested that a weak secretion of urate also occurs in the distal tubule. We have examined the site of urate secretion in the rabbit by the stop-flow method and with the aid of labelled urate.

The experiments were conducted on male animals, anaesthetized intravenously with 45 mg 'Nembutal'/kg. Respiration was maintained throughout the experiment by a respiration pump, and polyethylene catheters were inserted in the jugular vein and carotid artery on one side, and in the right ureter. The stop-flow procedure was similar to the method of Malvin *et al.*[5]. An infusion containing creatinine (0·5 per cent), mannitol (5 per cent), and in some instances urate (0·1 per cent), dissolved in physiological saline, was given through the venous cannula at a rate of 2·3 ml./min. One minute before releasing the occlusion, a solution containing 250 mg inulin and either 50 mg urate or 15 μc. 8-^{14}C-urate was injected over a period of 30 sec. Urate was analysed by an enzymatic method[6]. Radioactivity was measured in a Packard 'Tri-Carb' scintillation counter after dissolving 50 μl. of urine in 5 ml. of Bray's mixture[7].

Fig. 1 gives the results of an experiment in which 50 mg of urate was injected post-occlusively. The concentration of Na^+, urate, and inulin has been plotted as a function of the amount of urine collected after the stop-flow period. Corresponding to the dip in the Na^+-curve (which marks the distal part of the nephron), the urate concentration increases sharply. The concentration of urate reaches a peak value after collection of c. 4 ml. of urine. It can be seen from Fig. 1 that an increase in urate concentration occurs just before the appearance of inulin (the inulin indicates formation of new glomerular filtrate after the stop-flow period). The experiment thus shows a pronounced permeation of urate across the proximal tubules.

In the experiment shown in Fig. 2 urate was infused during the whole experiment, and probenecid—which inhibits urate secretion in the rabbit—was administered intravenously in a dose of 100 mg/kg prior to the stop-flow period. At the end of the occlusion period labelled urate was infused along with inulin. It appears from Fig. 2 that radioactivity in samples originating from the distal tubule is very small. An increased number of counts is

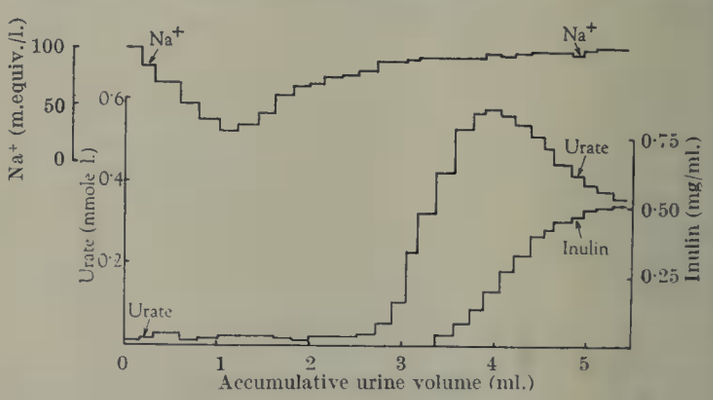

Fig. 1. The stop-flow pattern after injection of 50 mg urate and 250 mg inulin post-occlusively

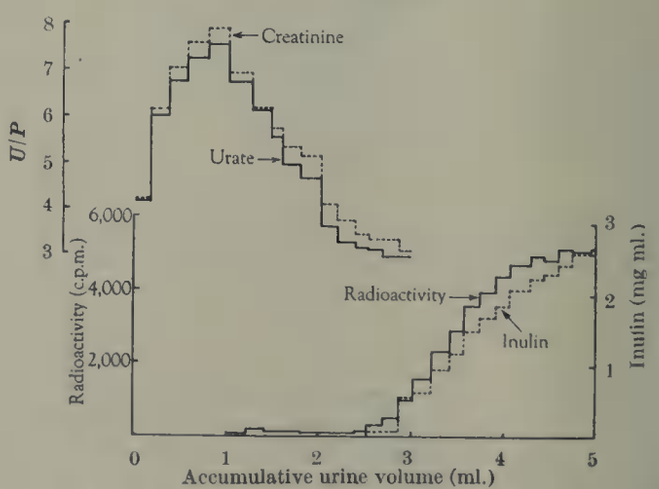

Fig. 2. The stop-flow pattern after injection of 15 μc. urate post-occlusively. Urate was infused during the whole experiment, and 100 mg probenecid was given intravenously prior to the stop-flow period

observed concomitantly with the excretion of inulin in the urine. It is seen that the rise in the 'radioactivity curve' is only slightly greater than that for the inulin curve. On the basis of these findings it may be concluded that probenecid strongly inhibits an active transport of urate from the plasma to the tubular fluid in the proximal tubules.

The two curves on the left side of the diagram show a plot of the U/P values of chemically determined urate and creatinine. A pronounced rise in the U/P values for urate and creatinine is observed in samples from the distal tubule. Creatinine may be used as an approximate measure of glomerular filtration rate in the rabbit, and the increase in the distal concentrations of creatinine during stop-flow conditions is due to re-absorption of water (following the re-absorption of Na^+). The U/P values for urate are a little below the corresponding ones for creatinine. A similar relationship between the excretion of urate and creatinine after administration of probenecid is found under free-flow conditions, and so it must be concluded that the re-absorption of water in the distal part of the nephron accounts entirely for the increased concentration of urate during the stop-flow period. No net transport of urate can be demonstrated, and since the distal tubule is practically impermeable to the radioactive urate, infused during the occlusion, urate has either moved out of or into the tubular fluid of the distal part of the nephron.

The experiments thus show that the secretion of urate in the rabbit is localized to the proximal tubules and may be almost completely inhibited by administration of probenecid. In contrast, the distal part of the nephron is virtually impermeable to urate.

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Response of Melanocytes of Dermis and Epidermis to Lightening Agents

THE frog has been prominent in investigations of hormonal control of human skin colour. The excised skin of *Rana pipiens* is sensitive to α-melanocyte stimulating hormone (α-MSH); less than 10^{-11} g added to 20 ml. of Ringer's solution containing the specimen produces darkening. The responsiveness of frog skin to MSH led to its use in an *in vitro* frog skin assay[1] which played an important part in the isolation and identification of α- and β-MSH[2]. These peptides when administered systemically darken human skin[3,4]. The use of the frog skin assay led also to the isolation of an indole, melatonin, which is synthesized in the pineal[5]. When added to frog skin previously darkened with MSH, melatonin was found to be a very potent lightening agent. Other compounds sharing this ability to lighten MSH darkened frog skin are norepinephrine and acetylcholine[6].

It is the purpose of this communication to contrast the effect of these lightening agents on dermal and epidermal melanocytes.

Frogs were decapitated, the skins removed and washed in four changes of frog-Ringer's solution for 3 h before use. Skins were mounted on rings, placed in beakers containing 20 ml. Ringer's solution and reflectance (skin colour) was measured with a 'Photovolt' model 610 reflectance meter as described[1].

Specimens were fixed in 10 per cent formol-saline for 30 min, then incubated with 0·5 per cent trypsin (1 : 250 Difco) for 10 min at 30°. The specimen was then rinsed and, under observation with a dissecting microscope, the epidermis was peeled from the dermis.

Examination of the epidermis in an area of a dark skin marking revealed contracted melanocytes with a rare projection (Fig. 1). Five units of MSH, added to the specimen 60 min before fixation, effected the expansion of epidermal melanocytes to arborized, dendritic forms with long-branched processes, morphologically indistinguishable from mammalian melanocytes (Fig. 2).

Melatonin (2×10^{-3} μg) added to a skin darkened for 60 min with 5 units of MSH produced almost complete lightening (increased reflectance) to a pre-MSH treatment value within 20 min. The epidermal melanocytes (Fig. 3), however, were identical with epidermal melanocytes of the MSH-darkened skin.

This lack of response of frog epidermal melanocytes to melatonin is consistent with its lack of effect on human skin colour[7] and guinea-pig epidermal melanocytes[8].

Dermal melanocytes from the preceding specimens were found to be contracted and compact before the addition of

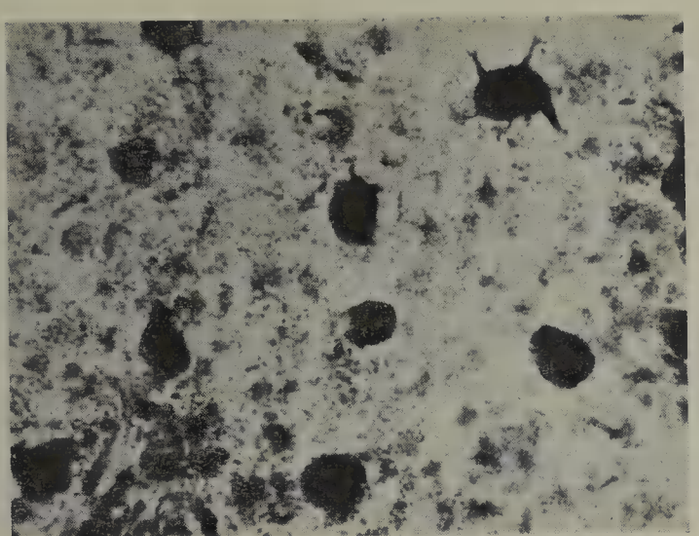

Fig. 1. Epidermis from skin of *Rana pipiens* which has been fixed and separated from dermis as indicated in text. The specimen was cleared in xylene and mounted on a glass slide. The skin was washed for 3 h in Ringer's solution before fixation. The melanocytes are dense and contracted. A rare dendritic process is filled with melanin granules. Notice the extra-melanocytic melanin granules in the epidermis. (×468)

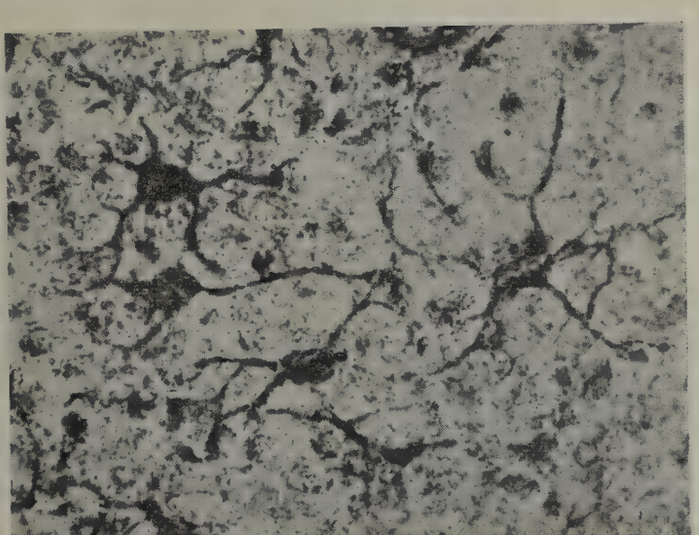

Fig. 2. Epidermis from a specimen exposed to 5 units of MSH for 60 min before fixation. The cell bodies are less dense than in Fig. 1. Melanin granules fill the delicate dendritic processes. (×468)

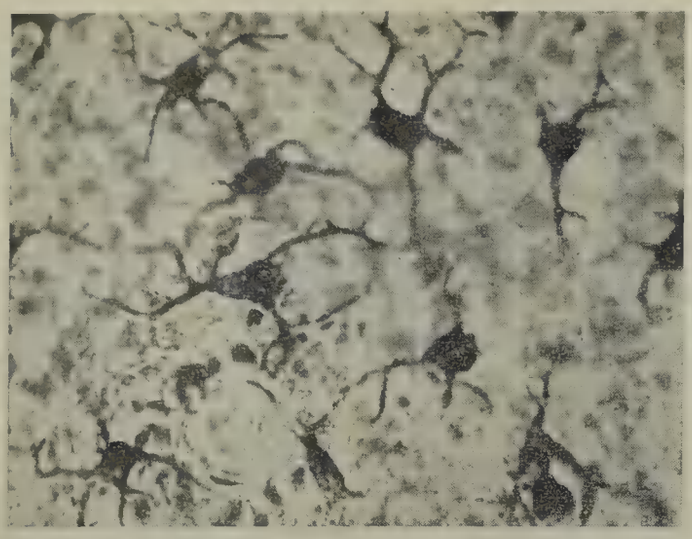

Fig. 3. Epidermis from a specimen exposed to 5 units of MSH for 60 min to which melatonin 2×10^{-3} μg was then added 20 min before fixation. The epidermal melanocytes are identical with those fixed in formalin before treatment with melatonin (Fig. 2). (× 468)

MSH. MSH effected the expansion of dermal melanocytes to stellate, dense forms. The addition of melatonin to the specimen darkened with MSH caused the dermal melanocytes to contract to the pre-MSH state.

Two other agents, acetylcholine and norepinephrine, are powerful lightening agents for MSH-darkened frog skin[6]. Acetylcholine (5×10^{-3} M) or norepinephrine (5×10^{-4} M) when added to specimens darkened with MSH produced 90 per cent lightening (increased reflectance) and contraction of the dermal melanocytes to the pre-MSH state. Neither acetylcholine nor norepinephrine, however, caused any morphological alteration in epidermal melanocytes previously expanded by MSH.

It is apparent from these observations that dermal melanocytes have a qualitatively different response to melatonin, acetylcholine and norepinephrine from epidermal melanocytes. Both populations of melanocytes respond similarly with melanin granule dispersion to MSH[9].

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Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein

Synthesis of the protein component of human low-density lipoprotein (LDL), and elaboration of these lipoproteins from lipid and protein constituents, are regarded as occurring in the liver[1]. In the Bassen-Kornzweig syndrome, absence of LDL is associated with steatorrhoea and changes in small-gut histology[2]. Salt *et al.*[3] have speculated that the gut wall might be one site of lipoprotein synthesis. Isselbacher and Budz[4] have shown that rat intestinal mucosa does synthesize lipoproteins. In rats, cholesterol synthesis occurs in the gut[5]. In human beings, 70 per cent of plasma cholesterol is carried by LDL, and if gut wall synthesis of cholesterol is shown to occur in man, the possibility again arises that a proportion of human LDL might also be elaborated in the intestinal mucosa. LDL newly synthesized by the gut could reach the general circulation via lacteal-lymphatic pathways, or directly through the portal venous system.

The opportunity to investigate possible synthesis and transport of LDL by the lymphatic pathway arose in two patients with chyluria secondary to filarial obstruction of upper abdominal and thoracic lymphatics. Lymphangiography in these patients had revealed free communication between distended lymphatics in the upper abdomen, including the area occupied by the base of the small gut mesentery, and the right renal pelvis. Proteinuria in the two patients averaged 13·0 g and 5·0 g a day respectively. This total included approximately 800 mg and 300 mg a day of LDL, determined by gel-diffusion precipitin assay[6], and by dextran sulphate precipitation[7].

Turnover of labelled LDL was followed in these patients by a method previously reported[8]. Total LDL was isolated from the serum of each patient, labelled with iodine-125 in the peptide component, and returned to the patient by intravenous injection. Serum LDL was assayed in duplicate by dextran sulphate precipitation, and the concentration of LDL in urine measured by a modified procedure using large volumes of urine and additional Ca^{++}. Specific activities were then calculated for plasma and for urine on the assumption that all radioactivity entering a trichloroacetic acid precipitate was associated with LDL. Data from the turnover investigations are shown in Table 1.

During the first 3 h of each investigation, a diuresis was produced by oral intake of fluid, including milk. In both patients, free radioiodide was present in urine passed 20 min after the intravenous injection, but protein-bound radioactivity was first detected in urine formed after 90 min in both patients. This interval of 90 min agreed with the time of appearance of T_{1824}-albumin in urine following intravenous injection of 6·78 mg of Evans blue into these two patients as a separate investigation, and contrasted with the rapid appearance of T_{1824}-albumin in patients with nephrotic syndrome. The time-courses for specific activity of plasma and urine are shown in Fig. 1. Specific activity figures for urine samples are plotted at the midpoint of the successive collection periods, which were short early in the investigations and over 24 h periods in the latter stages. Plasma specific activity declined steadily during the investigation. Urinary specific activity rose, during the 4–12-h (overnight) collection period, to reach a maximum value of the same order as that of the plasma, and then declined in parallel with plasma specific activity.

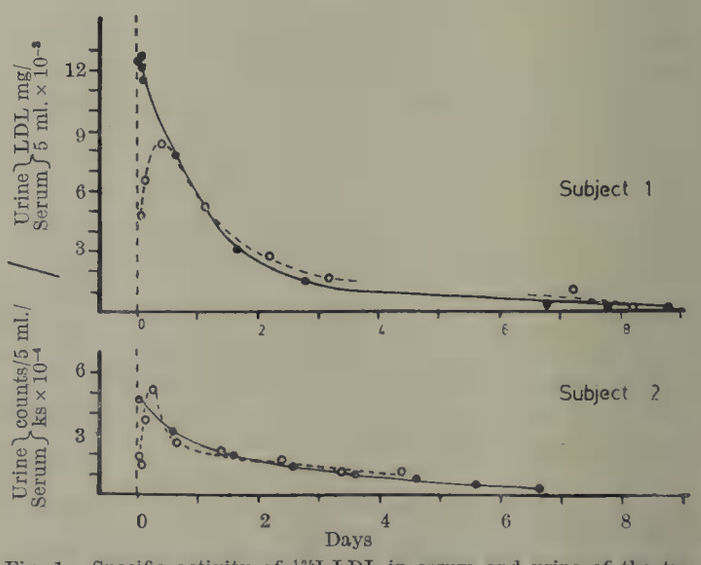

Fig. 1. Specific activity of ^{125}I-LDL in serum and urine of the two patients, expressed as counts per mg of total LDL in 5-ml. quantities of serum or urine. 24-h urine collections were incomplete in subject 1 over the interval 2·7–6·7 days. ●, Serum; ○, urine

Table 1. Turnover Results

Subject	Sex	Age	Wt. (kg)	Plasma volume (ml./kg)	Mean serum LDL level (mg/100 ml.)	Serum $t_{\frac{1}{2}}$ ^{125}I-LDL (days)	IV LDL pool (mg/kg)	U/P ratio (%)*	Corrected U/P ratio (%)†	Uncorrected catabolic rate serum LDL (mg/kg/day)‡	Corrected catabolic rate§	% of total ^{125}I-LDL distributed extravascularly¶
Patient 1	F	34	75	36·1	530	2·5	192	47·5	35·9	91·2	68·9	19
Patient 2	M	61	87	50·0	570	2·8	285	31·8	29·9	90·6	85·2	32
Range of values obtained in 7 normal men and 6 normal women for ^{131}I or ^{125}I-LDL studies		—	—	—	420 to 750	2·1 to 3·7	112 to 353	—	24·2 ot 54·4	—	44 to 138	12 to 40

* Ratio 24 h ^{125}I excretion to mean plasma radioactivity (ref. 12).
† U/P ratio recalculated, after correction for protein bound ^{125}I (^{125}I-LDL) in urine; that is, using only free ^{125}I excretion.
‡ Calculated from uncorrected U/P ratio.
§ Calculated from corrected U/P ratio.
¶ From multi-compartmental analysis by method of Matthews (ref. 12).

Turnover investigations have revealed a small extravascular distribution of LDL peptide in human subjects[9,10]. In animals there is evidence for transfer of LDL from plasma to hepatic lymph[11]. Human abdominal lymphatics may therefore contain some LDL from hepatic lymph besides transporting LDL which has passed into the extravascular compartment from blood capillaries. During a ^{125}I-LDL investigation, the fall in specific activity of plasma LDL is due to catabolism of labelled LDL with immediate excretion of iodine-125, and to dilution of the remaining ^{125}I-LDL by newly synthesized, unlabelled LDL. Regardless of catabolism, LDL returning to the blood vascular compartment via abdominal lymphatics would be expected to show the same specific activity as plasma unless newly synthesized lipoprotein was added to LDL in the lymphatics from gut tissues or possibly from the liver.

In our two patients specific activity in abdominal lymph rose over 4–12 h to equal that of plasma, and then maintained this equilibrium over successive 24-h periods. This finding argues against entry of newly synthesized LDL peptide into abdominal lymphatics. The results recorded here do not exclude LDL elaboration by the gut with direct passage of newly synthesized lipoprotein into the portal venous system. This possibility could be explored by the ^{125}I-LDL technique only if portal venous blood could be sampled over an adequate period and its specific activity compared with that of peripheral blood.

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Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body

Szentàgothai[1] reported the presence of axo-axonic endings on fibres entering the lateral geniculate body from the retina. Bizzi and Brooks[2] have shown that in the unanaesthetized, unrestrained cat irregular groups of waves could be recorded simultaneously from the pontine reticular formation and the lateral geniculate body during the desynchronized phase of sleep. The latter waves could no longer be recorded in the same conditions if the optic fibres were allowed to degenerate[3]. Recently, Andersen, Brooks, Eccles and Sears[4] have produced evidence pointing to the existence of presynaptic inhibition in the ventrobasal thalamus on stimulation of peripheral nerves.

Since axo-axonic endings have been demonstrated in the lateral geniculate body and the experiments by Bizzi and Boller[3] may be taken to indicate that the slow waves in the lateral geniculate body could be presynaptic in origin, an investigation has been carried out in order to see if there is any evidence for presynaptic inhibition in the same nucleus.

The experiments were performed on cats in which a complete transection of the brain stem immediately rostral to the exit of the trigeminal nerve had been made ('mid-pontine pre-trigeminal preparation'[5]) under ether anaesthesia. A micro-electrode was inserted into the lateral geniculate body and used to stimulate the optic fibres entering this nucleus. The antidromic response to this stimulus was recorded either from a bipolar concentric electrode inserted into the optic chiasm, or from the optic nerve after enucleation. The presence of any change in the terminal parts of the optic nerve fibres was assessed by any change in the excitability of these fibres near the microelectrode tip, and hence change in the size of the antidromic response[6].

It has been found that after a brief tetanus to the mesencephalic reticular formation or to the visual cortex the antidromic volley recorded either from the chiasm or from the optic nerve was increased in size. Examples of the increase in excitability of the optic nerve fibres after a tetanus of 8 shocks (with an interval of 3·2 msec) had been applied to the reticular formation and visual cortex are shown in Figs. 1*A* and *B* respectively. It can be seen that the maximum increase in excitability occurred around 50 msec after the start of the tetanus and the effect of the tetanus lasted for approximately 250 msec. In addition, after stimulation of the reticular formation with a short tetanus, a negative wave could be recorded from the optic nerve, which resembled the dorsal root potential[7]. This latter wave was found to increase in size with a decrease in the body temperature of the animal, and to decrease in size with an increase in the rate of presentation of the tetanus. An example of the slow wave recorded from the optic nerve to a tetanus of 8 shocks with an interval of 3·2 msec to the mesencephalic reticular formation is shown in Fig. 1*C*. Often, especially at low temperature, spikes were seen to be superimposed on the summit of the slow wave; these spikes could also be recorded from the optic chiasm. The spikes may correspond to the dorsal root reflex[8]. Comparison of Figs. 1*A* and *C* shows that the increase in excitability and the slow wave of the optic nerve ran a similar time-course. That this slow wave was probably not an envelope of

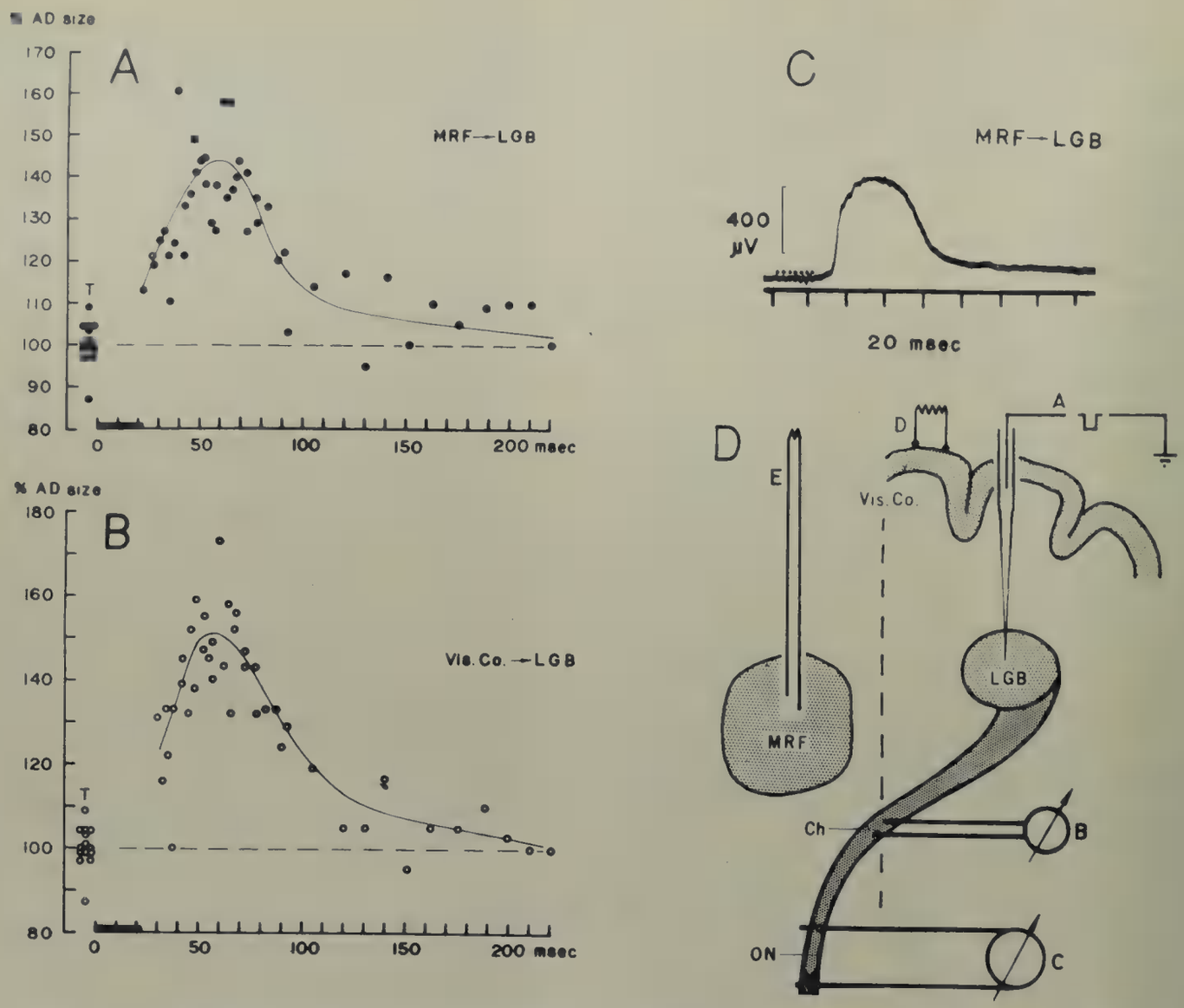

Fig. 1. Evidence for depolarization in optic nerve fibres by stimulation of the mesencephalic reticular formation and visual cortex. The experimental arrangement is shown diagrammatically in *D*. The micropipette used for stimulating is placed in the lateral geniculate body (*LGB*); the electrodes for recording the antidromic response were either in the chiasm (*Ch*) or on the optic nerve (*ON*). The graphs show the effect on the percentage amplitude of the antidromic response (*AD*) at various times after a brief tetanus (8 shocks with a duration of 1 msec and a separation of 3·2 msec) applied at a rate of 1/5 sec to the mesencephalic reticular formation (*A*) and the visual cortex (*B*). Each point is the average of 5 observations. The smooth curves were obtained by averaging the observations in each 50 msec period and drawing the curves through these points. Both graphs were obtained from the same animal in which the stimuli were applied alternately to the mesencephalic reticular formation and the visual cortex. The slow wave recorded monophasically from the optic nerve after stimulation of the mesencephalic reticular formation in an animal with a rectal temperature of 32° C is shown in *C*

centrifugally conducted impulses has been shown by stimulating the chiasm with a single electric shock at various times during the slow wave: no evidence of occlusion between the action potential, evoked by the chiasm stimulus, and slow wave was obtained.

Using the size of the slow wave as an indicator of the effectiveness of the tetanus applied to the brain stem, it has been found that the regions giving the biggest slow wave are centred around the central tegmental tract.

The increased excitability of the optic fibres, produced by stimulation of the mesencephalic reticular formation and visual cortex, can be taken to indicate that both structures exert a presynaptic inhibitory control on the transmission through the lateral geniculate body, according to previous investigations[11]. The possibility that the increased excitability of the optic nerve fibres may be passively produced by current flowing in a potential field due to activity elicited in nearby structures exists and cannot be overlooked. However, the fact that the time-course of the increased excitability, and of the slow wave, in the optic nerve have the long duration characteristic of the presynaptic inhibition shown in the spinal cord, and cuneate nucleus after cortical stimulation[9,10] seems to indicate that the phenomenon we observe is due to a true presynaptic inhibition.

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HAEMATOLOGY

Chronic Granulocytic Leukaemia : Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells

An abnormal marker chromosome in chronic granulocytic leukaemia was described by Nowell and Hungerford[1,2] and its occurrence widely confirmed[3–7]. This marker, the Philadelphia chromosome (Ph¹), occurs in almost all typical cases of chronic granulocytic leukaemia and appears to be an acquired abnormality specific for the disease. The Ph¹ is derived from a chromosome of pair 21 by loss of a variable proportion of the long arms.

It has been shown[8] that in untreated chronic granulocytic leukaemia all the dividing cells in direct preparations of bone marrow are Ph¹-positive. Apparently, megakaryocytes and erythroid precursors as well as granulocyte precursors carry the Ph¹. As haematological abnormalities are usually confined to the granulocytic series, it has been suggested[9] that the genetic material lost from the Ph¹ has immediate relevance to leucopoiesis only. It is postulated[8] that the erythroid, granulocytic, and megakaryocytic series have a common stem cell and the original deletion to produce the Ph¹ occurs in this stem-line. However, peripheral blood cultures in untreated cases yield mixed populations of Ph¹-positive and negative cells[4,11]. As the dividing cells in cultures of normal blood are almost certainly of lymphoid origin[10], it appears that the blood lymphocytes are Ph¹-negative and therefore must originate from a separate stem-line. Apart from cells of bone marrow origin and lymphocytes in the peripheral blood, cultured subcutaneous fibroblasts have been examined for the presence of the Ph¹ chromosome with negative results[3]. Only in the spleen are cells of both lymphocytic and bone marrow stem-lines to be expected to occur together, and so it is particularly important to study the cytogenetics of that organ in chronic granulocytic leukaemia. To our knowledge no such investigations have been reported. We have developed a method[12] for the cytogenetic study of lymphoid tissue; this technique was applied to spleen to obtain the results here reported.

The patient was a married woman, aged twenty-nine. Chronic granulocytic leukaemia, diagnosed 24 months previously, had been treated with busulphan, splenic irradiation, 6-mercaptopurine and cyclophosphamide. Resistance to all therapy had arisen and the disease had undergone acute transformation. Splenectomy was performed for massive splenomegaly and intractable thrombocytopenia. Splenic cell suspensions were cultured: (*a*) for 16 h without phytohaemagglutinin, (*b*) for 84 h with added phytohaemagglutinin. The chromosome count distribution was:

Chromosome No.	< 45	45	46	47	48	92–93	Totals
No. of cells (*a*)	7	8	26	5	1	0	47
(*b*)	7	12	53	16	0	2	90

On analysis, the dominant cell line was diploid and Ph¹-positive. A second line with 47 chromosomes due to the presence of a second Ph¹ was also identified. Excluding technically unsuitable cells, results of analysis as regards the presence of the Ph¹ chromosome were:

	Cells analysed	Ph¹ +	Ph¹ + +	Ph¹ −	Status uncertain
Preparation (*a*)	41	29	7	1	4
(*b*)	73	57	14	1	1

Thus Ph¹ negative cells accounted for only 2·4 per cent of the cells in 16-h culture and 1·4 per cent in 84-h culture. Splenic sections showed loss of normal architecture and absence of lymphocyte aggregations. A differential count of 500 cells in a splenic imprint showed 4 per cent of lymphocytes in a population of leukaemic blast cells and promyelocytes.

Thus almost all dividing cells from this patient's spleen were Ph¹ positive. This result, to be expected in the 16-h unstimulated culture, is surprising in the 84-h culture with phytohaemagglutinin, where Ph¹-negative cells of lymphoid origin might well be prominent. Possibly these cells failed to divide; however, splenic lymphocytes have been successfully cultured in this laboratory and elsewhere[13]. The initial small number of lymphocytes relative to leukaemic blast cells might explain the rarity of Ph¹-negative mitoses, but studies have shown that in cultures with phytohaemagglutinin normal cells may outstrip leukaemic cells[14–16]. A better hypothesis is that in advanced chronic granulocytic leukaemia all splenic stem cells, possibly including lymphocytic precursors, carry the Ph¹.

The presence of two Ph¹ chromosomes per cell has previously been observed in the chronic phase of chronic granulocytic leukaemia[17] and also in the phase of acute transformation[18–20], when it is more common. Further investigations of splenic cytogenetics in chronic granulocytic leukaemia during the chronic phase and after acute transformation are desirable.

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Stable Messenger RNA in Nucleated Erythrocytes

The mammalian reticulocyte is capable of synthesizing haemoglobin for many hours after its nucleus has been extruded. Since the nucleus is the site of messenger-RNA synthesis[1], these cells must be using stable messenger for continued protein synthesis. In contrast to the red blood cells of mammals, erythrocytes of lower vertebrates and invertebrates are almost always nucleated. With the nucleus present these cells could conceivably synthesize protein on short-lived messenger templates. In order to determine whether nucleated red blood cells utilize short- or long-lived messenger-RNA, representative avian and reptilian erythrocytes were investigated by stopping DNA-dependent RNA synthesis with actinomycin *D* and following the course of protein synthesis in the functionally 'enucleated' cells.

Reticulocytosis was produced in young chickens and in adult turtles (*Pseudemys elegans*) by repeated bleedings; 15-day chick embryo erythrocytes (about 100 per cent reticulocytes) were used with identical results. Reticulocytes in turtle blood samples numbered about 10 per cent and in chick blood about 30 per cent. The red cells were separated by centrifugation, washed, and incubated in Waymouth medium with penicillin and streptomycin in

a 5 per cent atmosphere of carbon dioxide. Chick cells were incubated at 37°, turtle cells at 30°. Parallel incubations were done with control red cells and with red cell mixtures containing 10 μg/ml. actinomycin *D*. Aliquots were removed at intervals, washed, and incubated for 30 min with 5 μc./ml. L-leucine-^{14}C (sp. act. 220 mc./mM) in saline. The cells were precipitated with 5 per cent trichloroacetic acid (TCA), plated on 'Millipore' filters and counted in a gas flow counter. Each experiment was repeated at least twice. To ensure that actinomycin stopped messenger-RNA synthesis persisting in circulating cells, experiments were performed in which reticulocytes were incubated in nutrient medium with 0·5 μc./ml. uridine-^{14}C (sp. act. 25 mc./mM) for 10 min. The culture was then divided and 10 μg/ml. actinomycin added to one portion. The synthesis of RNA (uridine counts precipitable with cold 5 per cent TCA) was shown to stop within 10 min for chick cells and within 20 min for turtle cells.

Experiments such as those in Fig. 1 demonstrated that protein synthesis continued for at least 24 h in the absence of new messenger-RNA production. The life-span of the messenger was similar in both the chicken and in the turtle erythrocytes. The results do not show that protein synthesis in these red blood cells is equivalent to haemoglobin synthesis, but haemoglobin is the major protein product of the mammalian reticulocyte[2], and it is likely for these cells as well.

The fact that there is long-lived messenger-RNA in the chicken and turtle erythrocytes plus the evidence of Cameron and Prescott[3] that maturation of the chick erythrocyte nucleus is associated with cessation of RNA synthesis indicate that in the maturing erythrocyte the nucleus is inactive so far as transfer of genetic information is concerned.

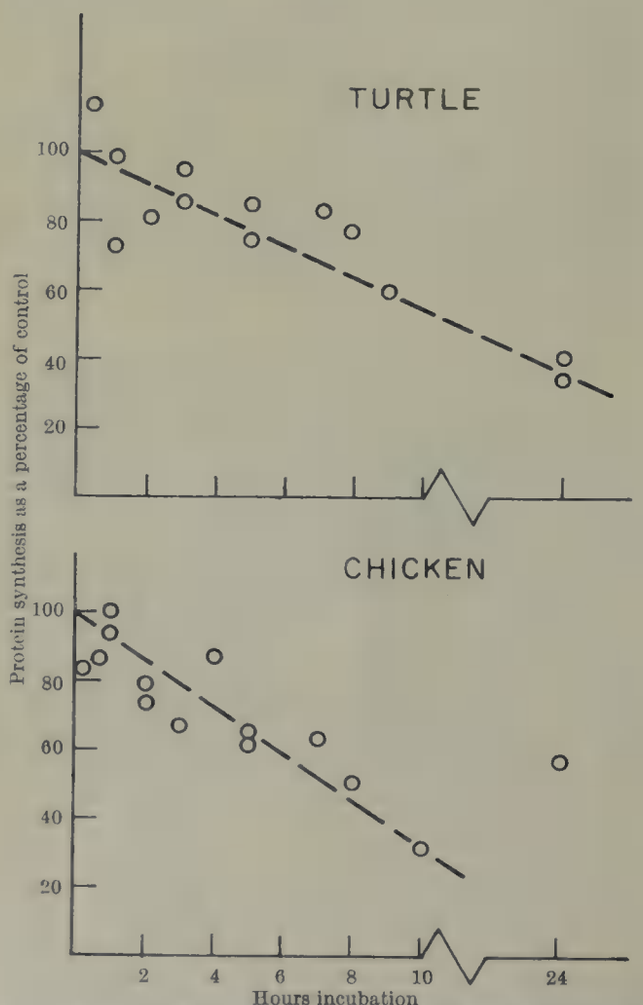

Fig. 1. Persistence *in vitro* of protein synthesis (above) in turtle erythrocytes (incubated at 30°) and (below) in chicken erythrocytes (incubated at 37°) after stopping messenger-RNA synthesis with actinomycin *D*

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IMMUNOLOGY

Serological Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides*

A GALACTAN (pneumogalactan) isolated from bovine lung[1,2] precipitated 28 per cent of pneumococcal polysaccharide type XIV antiserum[3]. This suggested that both polysaccharides have galactose residues of similar linkages, possibly β-1:6, β-1:3 or β-1:3:6[4], and agreed with the postulated structures of type XIV pneumococcal polysaccharide[4] and of lung galactan[2].

Recently it was reported[5,6] that a galactan was isolated from *Mycoplasma mycoides*, the causative organism of contagious bovine pleuropneumonia (CBPP). It was decided, therefore, to determine whether a serological relationship existed between the galactans from normal bovine lung and from this organism causing lung lesions in cattle.

Purified pneumogalactan was kindly supplied by Hoffman-La Roche, Inc., Nutley 10, New Jersey. Since the isolation method of pneumogalactan is not described, we extracted, with hot phenol[7], an acetone powder of bovine lung obtained from the U.S.A., a country free of CBPP (California Corporation for Biochemical Research, 3625 Medford St., Los Angeles); this material is referred to as normal bovine lung extract. *M. mycoides* cells were used for preparation of antiserum (*x'*) in sheep[8]. Sera from animals naturally infected with CBPP were also used (sera Nos. 21 and 42). As our attempts to produce antisera against pneumogalactan have so far failed, the results presented here are of experiments with pneumogalactan, *M. mycoides* antigens and antisera against *M. mycoides*.

Table 1 shows the results of complement-fixation, indirect haemagglutination[9] and tube agglutination tests, using *M. mycoides* antigen with immune sera and sera absorbed with excess pneumogalactan. These results show that the pneumogalactan absorbs a considerable proportion of the relevant antibodies against *M. mycoides*.

In the agar-gel precipitin test, serum against *M. mycoides* (*x'*) gave homologous lines with hot phenol-extracted carbohydrate from *M. mycoides*[10], pneumogalactan, and normal bovine lung extract. Sera 21, 42, and *x'* formed precipitin lines with hot-phenol-extracted carbohydrate: after absorption with 30 mg of pneumogalactan no lines were formed with either serum 21 or 42 and only very faint lines were produced with serum *x'*.

Table 1. RECIPROCAL TITRES OF *M. mycoides* ANTISERA, BEFORE AND AFTER ABSORPTION WITH PNEUMOGALACTAN, AGAINST *M. mycoides* ANTIGEN

M. mycoides antiserum	Complement fixation	Indirect haemagglutination	Agglutination
x' before absorption	80	640	640
after absorption	10	80	80
'21' before absorption	80	320	80
after absorption	20	80	40
'42' before absorption	160	320	320
after absorption	20	80	40

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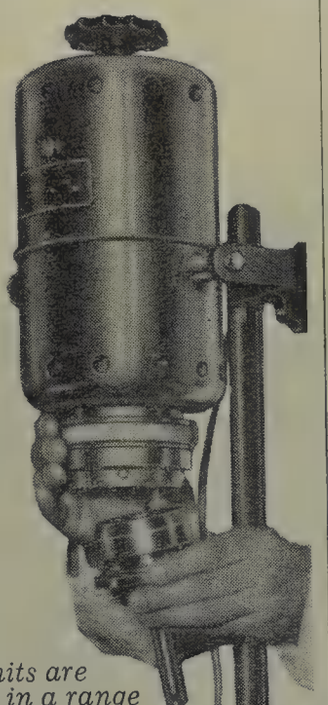

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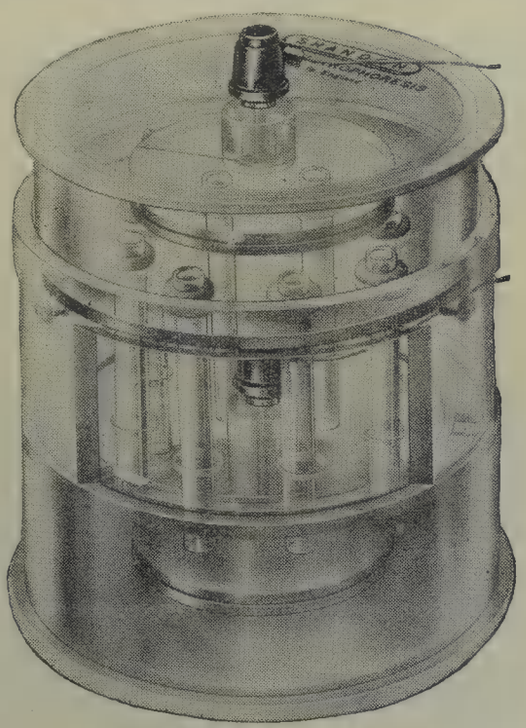

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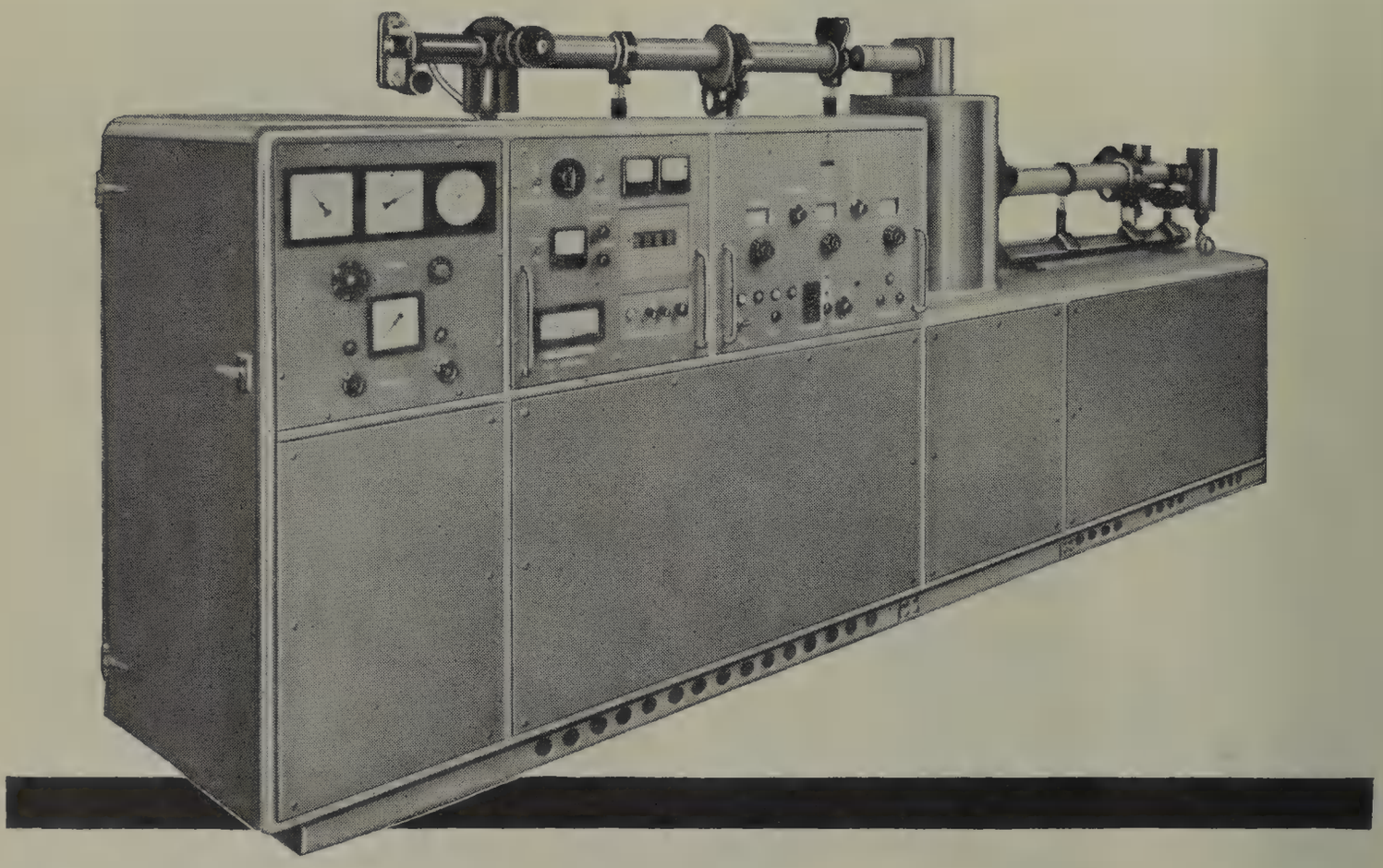

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Animals naturally infected with CBPP (Nos. 21 and 42) gave positive skin test reactions[11] with both hot-phenol-extracted carbohydrate from *M. mycoides* and pneumogalactan.

The cross-reactivity of pneumogalactan and the galactan from *M. mycoides* indicates that both these polymers have some common linkages. We are now investigating the possibility that the pneumogalactan plays a part in the pathogenesis of CBPP, either in an allergic type reaction, or by localizing the organisms in the lung through an antibody–antigen reaction. It would also be of interest to determine whether human lung contains a galactan which might cross-react with the pneumococcal polysaccharides.

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RADIOBIOLOGY

Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver

Blyumenfel'd and Kalmanson[1] as well as Gordy[2] and Miyagawa[3] have already reported that radicals are formed in lyophilized rat liver by ionizing radiation using electron spin resonance techniques. Moreover, Gordy and Miyagawa have shown that oxygen and cysteine, which was allowed to diffuse into the liver before freeze-drying, alter the electron spin resonance spectra. This report describes investigations on X-irradiated mouse liver. The study also includes preparations of liver in which solutions of cysteine were injected before freeze-drying. To some extent we obtained results different from those described by the foregoing authors.

Livers freshly removed from mice were frozen at 77° K in an untreated state and subsequently lyophilized. Preparations containing cysteine were obtained by injecting 0·5 ml. of a cysteine solution (300 mg cysteine-HCl/ml. H_2O) into the freshly-removed organ. Then the liver was frozen at 77° K and lyophilized. The preparations were ground (bulk volume about 330 cm³/100 g) and irradiated with X-rays (200 kV, half-value layer: 0·64 mm copper, dose-rate: 920 r./min, dose: 100 kr.) under vacuum (about 10^{-3} torr) and in air respectively at room temperature. The electron spin resonance measurements were carried out under the same conditions, with an X-band spectrometer. Technical details were as already described in a previous publication[4].

Samples of non-irradiated liver show a signal (5×10^{16} radicals/g), which possesses the structure of an unresolved doublet. The 'blank' signal looks like the dotted spectrum

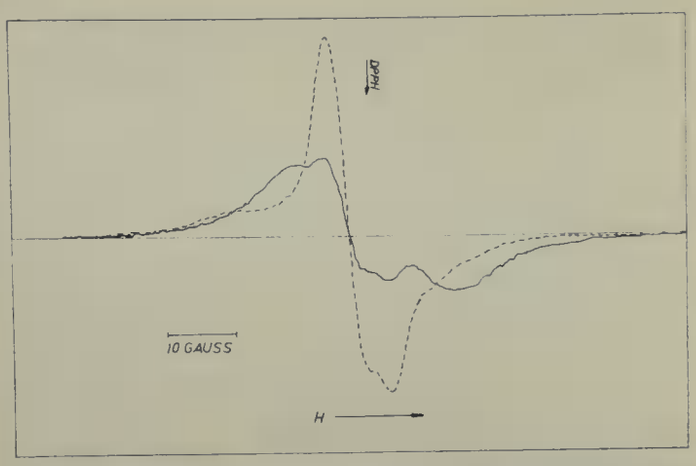

Fig. 1. Electron spin resonance spectra of X-irradiated lyophilized liver. The solid line gives the resonance of a sample irradiated and measured in vacuum, and the dotted line shows the resonance of the same sample measured 20 min later after admission of air

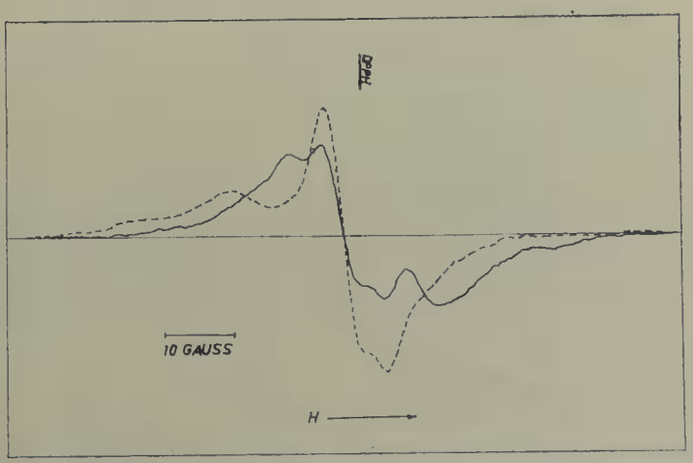

Fig. 2. Electron spin resonance spectra of X-irradiated lyophilized liver treated with cysteine. The solid line gives the resonance of a sample irradiated and measured in vacuum, and the dotted line shows the resonance of the same sample measured 20 min later after admission of air

in Fig. 1 but does not correspond with the glycylglycine-type signal[5] because of its smaller breadth. A touch of moisture leads to a rapid decay of the blank. Irradiation of the liver preparation (with and without cysteine respectively) in air produces an increase of the 'blank' signal without a change of its structure.

Irradiations of the samples with and without cysteine respectively under vacuum conditions produce in both cases the same electron spin resonance spectra (compare the solid spectra in Figs. 1 and 2). The 'blank' signal is slightly increased by the irradiation and overlapped by a second broad signal. With regard to the 'blank' signal the radiation yield amounts to $G = 3 \cdot 0$ per 100 eV absorbed X-ray energy. The increase of the doublet by irradiation may be due to oxygen residues in the sample tube.

An admission of air to the substances irradiated under vacuum immediately leads to a change of the electron spin resonance spectra. The alteration produces somewhat different spectra in the samples with and without cysteine (see dotted spectra in Figs. 1 and 2). In both cases the broad signal is quenched and at the same time the doublet is increased. This process continues for some hours after admission of air, because the air has to penetrate the whole sample by diffusion. Immediately after admission of air only about 80 per cent of the initial radical concentration can be measured. In the preparation with cysteine the increase of the doublet is not so large as in that without cysteine. On the other hand, an additional signal is formed in the region of lower magnetic field strength. This may be due to a sulphur radical, although it is not the typical 'cysteine-cystine-spectrum'.

Warming the irradiated sample in the evacuated state to 353° K for 10 min will only quench the broad signal; the signal height of the doublet is not altered all the time.

Only if the sample is stored for several hours at this high temperature does the radical concentration of the doublet also decrease.

These investigations show that atmospheric oxygen is able to induce a radical shift without external energy feed. Similarly, we have also found a transposition of X-ray-induced radicals from a carbon to a sulphur atom in a dimeric form of homocysteine-thiolactone, in experiments which we hope to report soon.

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'Cell Killing' in Radiobiology

One of the most significant advances in cellular radiobiology was the development by Puck and Marcus[1] of a technique by means of which the survival from irradiation of animal cells in culture was placed on a quantitative basis. Survival has usually been expressed arbitrarily as the ability of an irradiated cell to produce, over a 10–20-day period, a macroscopic clone of descendants comprising at least 50 'normal' (that is, non-giant) cells.

The loss of this capability is at present described in a variety of ways: 'cellular death', 'cellular lethality', 'reproductive death', or 'reproductive incapacity'[2]; 'inhibition of clone formation', 'damage to reproductive capacity', or 'inhibition of unlimited proliferation'[3]; 'lethal events' or 'killing'[4]; 'reproductive cell killing', 'loss of reproductive integrity' or, 'cell killing'[5]; 'effect on ability to proliferate'[6]; 'loss of proliferative capacity'[7]; 'loss of proliferative integrity', or 'lethality'[8].

May one now enter a plea for a return to the succinct terminology of Puck and Marcus? These authors pointed out the analogy between their technique with irradiated HeLa cells and those with irradiated bacteria. They also showed that the term 'cell killing' had long had a specific meaning in microbiology, referring only to the ability of an individual cell to produce a macroscopic colony. In the absence of full knowledge of the mechanisms involved in the prevention of clone formation by irradiation, it is difficult to describe the phenomenon without ambiguity. But the use of a standard phrase in all reports of radiobiological investigations involving single cell plating techniques would seem to be a step in the right direction; and 'cell killing' is proposed as being the most suitable choice.

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BIOLOGY

Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography

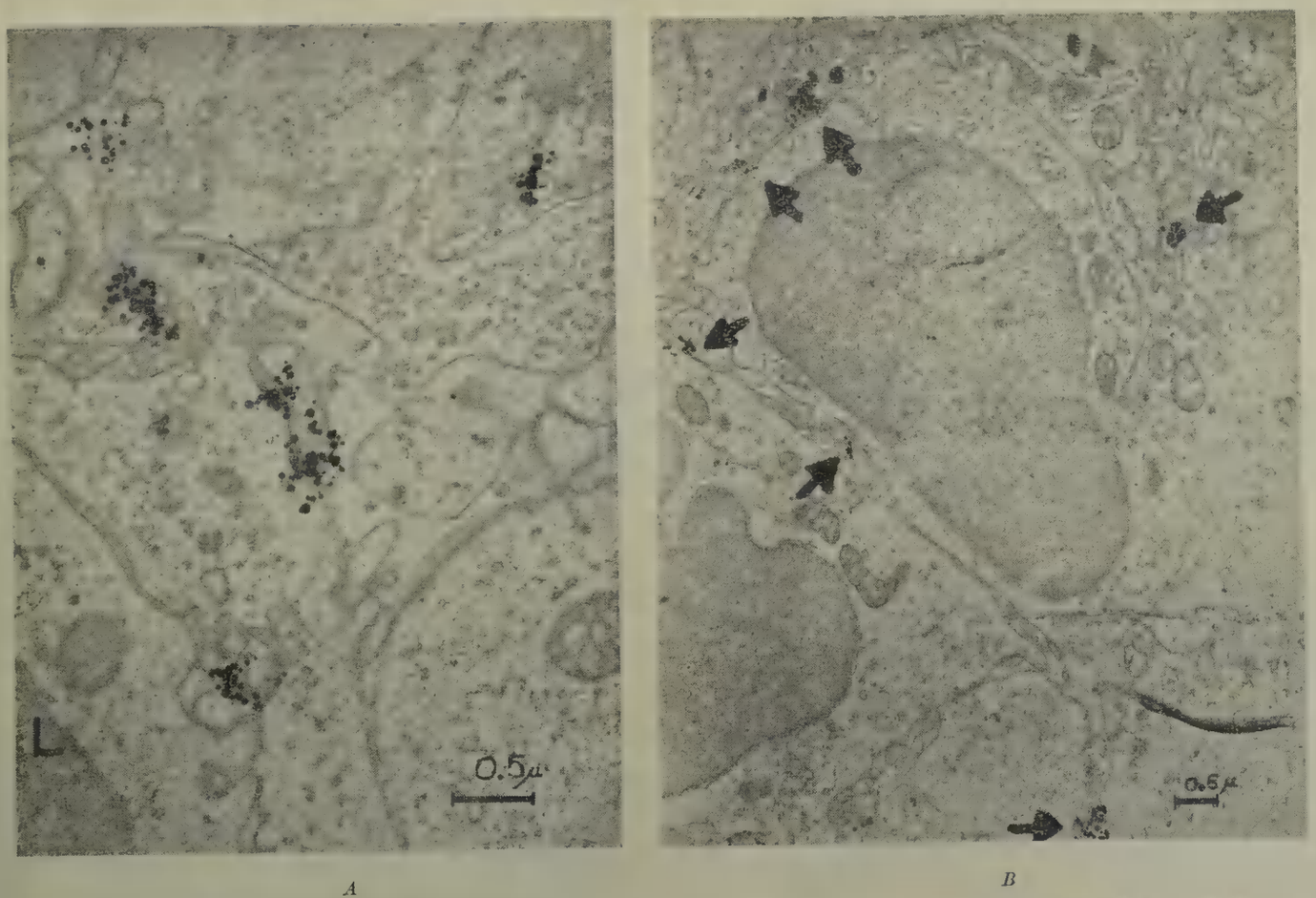
Previous investigations in this laboratory[1–4] have described the localization of antigens in lymphoid follicles. Much circumstantial evidence was presented for the existence in follicles of a highly specialized fine dendritic web of macrophage fibrils responsible for antigen trapping and retention. Light microscopy alone, however, proved inadequate for the unequivocal demonstration of the structure of this web. The electron microscopic investigation reported here has defined more precisely the sites of localization of *Salmonella* flagellar antigen in the rat popliteal lymph node.

Whole flagella of *Salmonella adelaide* were labelled *in vitro* with iodine-125 by the direct oxidation method of Hunter and Greenwood as has been described elsewhere[5]. Ten-week-old normal Wistar rats were injected in one hind-foot pad with 20 μg flagella containing 1·0 mc. iodine-125 (0·7 g atom iodine-125 per 30,000 g flagella). The draining popliteal lymph node was removed one to five days later, diced into pieces of about 2 mm^3, fixed in cold buffered OsO_4 (ref. 6), dehydrated in ethanol and embedded in 'Araldite'. Throughout the fixation and dehydration, less than 5 per cent of the node's total radioactivity was lost[7], despite the large area of exposed cut surfaces.

A section 2μ thick, embracing the total available area of tissue, was cut from each block and mounted on a gelatine-coated slide for normal autoradiography with Kodak *NTB*-2 emulsion. To facilitate subsequent staining with methyl green-pyronin, the 'Araldite' was removed from the section with sodium methoxide[8] before the emulsion was applied. Exposure for one day was sufficient to allow the detection of labelled follicles under 100-fold magnification. The position of a heavily labelled follicle was noted on the autoradiograph, the 'Araldite' block oriented appropriately and then trimmed to the size of the specified area comprising a follicle and some perifollicular tissue. Other blocks were prepared to contain typical areas of heavily labelled lymph node medulla. Ultrathin sections displaying silver to pale gold coloration were cut with glass knives on a Huxley ultramicrotome and mounted on collodion coated slides[9]. Matching sections (0·5μ) were cut to enable precise light microscope autoradiographic correlation studies to be made.

The technique of high-resolution autoradiography developed by Salpeter and Bachmann[9] with Kodak *NTE* emulsion was used with the following modifications: (1) the slides were fire-polished[10] before being coated with collodion; (2) sections were either unstained, or were stained for 0·5–2 h (before or after mounting) with 5 per cent aqueous uranyl acetate; (3) the emulsion was applied by dipping the slides into a Coplin jar containing diluted[9] *NTE* emulsion. After exposure for 1–3 months, the slides were developed by passing them through the following solutions: 'Dektol' (Kodak, diluted 1/2) at 24° C, 1 min; 3 per cent CH_3COOH, 10 sec; water, 5 sec; 'Amfix' (May and Baker, Australia), 30 sec; water, 2 min. Finally, the slides were soaked in distilled water for 1 h or overnight before the collodion film was scored around the area of the sections and then stripped from the glass. Sections were mounted on either annular copper disks of 1-mm internal diameter[11] or *NEW* 100 'Athene' grids (Smethurst High-Light, Ltd., England). A Siemens *UM*100*E*4 electron microscope was used with a 50-μ objective aperture and was operated at 60 kV. Specimens were photographed at magnifications ranging from × 1,250 to × 7,200.

The specificity of the developed grains was established by locating the label on serial sections. With the development régime used, the grains appeared as round or



A *B*

Fig. 1. Electron microscopic autoradiographs of a primary follicle from the popliteal lymph node of a rat injected with ^{125}I-labelled *Salmonella* flagella. *A*, Note the heavy label associated with fine cytoplasmic processes. *L*, Lymphocyte. (×*c.* 19,260). *B*, Magnification × 11,300. A lymphocyte surrounded by membrane-associated clumps of label

comma-shaped dots (diam. 400 ± 200 Å), rather than as spirals. Significant background labelling was not seen, so that two or more grains at the one location could be identified with some confidence as specific label. By making a photo-montage of the complete section, the exact nature and location of labelled cells could be correlated with the more familiar and characteristic appearance of the matching autoradiograph in the light microscope.

The results showed that label in primary lymphoid follicles was indeed associated with fine cytoplasmic processes lying between the lymphoid cells (Fig. 1), thus confirming the previous findings and speculations[4]. The processes appeared to be long, fine, convoluted extensions of dendritic reticular cells. The label showed close association with the cytoplasmic membrane of these processes, a fact that was more readily apparent from inspection of areas where the density of grains was lower than in Fig. 1. The lack of label of the body of reticular cells and of the broader processes emanating from them (Fig. 1*A*) suggested that the antigen was preferentially located at the terminal arboreal regions of the dendritic processes.

The association between labelled dendritic processes and adjacent lymphocytes was extremely close (Fig. 1*B*). In some cases the contact was so intimate that it was impossible to determine whether label emanated from the process of a reticular cell or from the cytoplasmic membranes of the lymphocyte itself. The labelled dendritic processes were distinct from the cytoplasmic extensions which often ensheathed clumps of collagen fibrils. Furthermore, the dendrites did not appear to emanate from the 'tingible body' macrophages[12] which contained no obvious intracellular label.

The findings in the lymph node medulla were strikingly different. Here the label was located almost exclusively in the cytoplasm of typical macrophages (Fig. 2), much of it being associated with large granules and vacuoles. There was no evidence of label specifically associated with cytoplasmic processes or cell membranes.

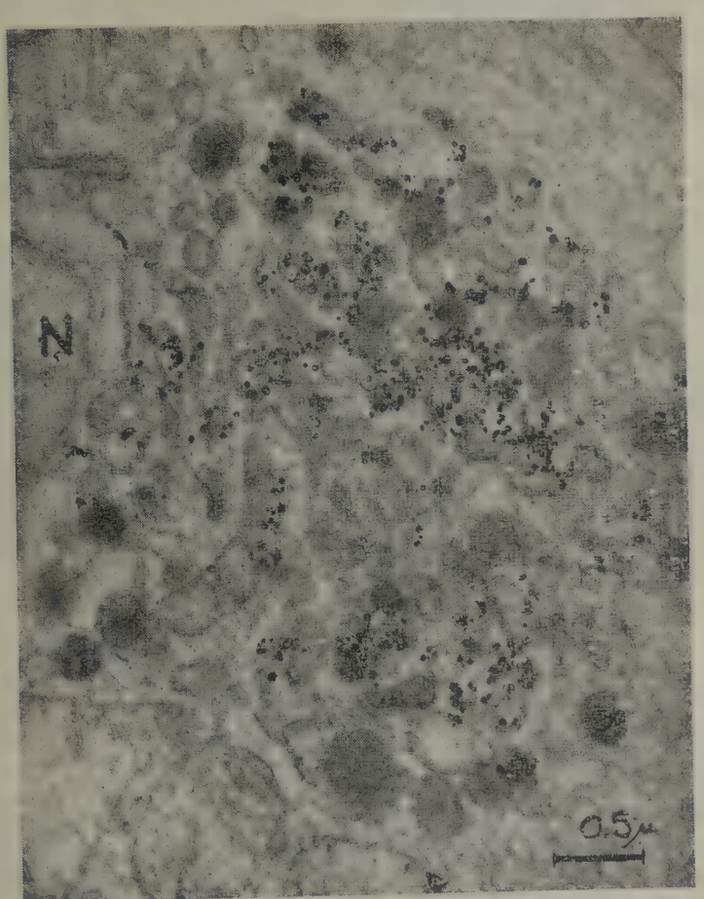


Fig. 2. Portion of a medullary macrophage showing intracellular label predominantly associated with electron dense granules. *N*, Nucleus of the macrophage. (×*c.* 19,300)

We conclude that there are two basic mechanisms for antigen retention in lymph nodes. In the lymphoid follicles there is a mechanism whereby antigen becomes

associated with the surface of dendritic processes of reticular cells, where it comes in contact with lymphocytes. By contrast, in the medullary sinuses antigen is retained intracellularly in the inclusions of typical phagocytic cells. The distribution of label in lymphoid follicles leads us to suggest that the term 'phagocytic reticulum' which we have previously used[4] in relation to this area should be replaced by the term 'antigen-retaining reticulum'. It is tempting to speculate that the two patterns of lymph node antigen distribution reflect different facets of the immune processes.

We thank Prof. G. J. V. Nossal and Dr. G. L. Ada for their advice and Mr. J. Pye for performing the iodinations. This work was supported by grants from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service (*AI*–0–3958), to Prof. Nossal, and from the National Health and Medical Research Council, Australia. Some batches of iodine-125 were a gift of Glaxo Laboratories, England.

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Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm.

Some inorganic salts stimulate eggs of the beet cyst nematode, *Heterodera schachtii* Schm., to hatch, but less effectively than diffusate from sugar-beet roots[1–3]. The effective ions are potential oxidizing agents. We have extended hatching tests to some metallic ions not previously tried, including Ba^{2+}, Al^{3+}, Pb^{2+}, MoO_4^-, Mn^{2+}, Co^{2+}, Zn^{2+} and Cd^{2+}, and have re-tested some salts ($MgCl_2$, KCl, NaCl, $HgCl_2$ and $FeCl_3$). Copper, previously tested as the sulphate, was tested as the chloride, and chlorides of the other metals were included in tests wherever possible. All salts were first tested at a concentration of ~3 mM in water and those with some activity were tested again over a range of concentrations. The hatch of eggs (Table 1) is expressed as a hatch rating[3]:

$$\frac{H_s - H_w}{H_d - H_w} \times 100$$

where H_s is the hatch in the substance, H_d is the hatch in beet root diffusate and H_w is the hatch in distilled water, and also as a percentage of the total number of eggs in the cysts.

Zinc sulphate, zinc nitrate and zinc and cadmium chlorides proved potent hatching agents; twelve other salts were moderately or weakly active, and ten were inactive or inhibitory. The zinc salts and cadmium chloride are more effective than the other inorganic hatching agents for *H. schachtii*[3] although neither anion nor cation is a potential oxidizing agent. The activity of these two chlorides (zinc and cadmium) was not caused by hydrochloric acid formed by hydrolysis, because hydrochloric acid itself is only moderately active at its optimum concentration.

Table 1. The Ability of Various Metallic Salts to stimulate Hatching of *H. schachtii* Eggs expressed as a Hatch Rating and also as a Percentage of the Total Number of Eggs in the Cysts

Based on total hatch after three weeks from three batches of 100 cysts

Compound	Concentration (mM)	Hatch rating	Percentage hatch
Zinc chloride	4*	**180**†	64
Cadmium chloride	0·6*	**177**	56
Zinc sulphate	2	**127**	55
Zinc nitrate	2	**113**	45
Ammonium molybdate	3*	82	38
Lead acetate	3*	75	23
Aluminium chloride	3*	69	32
Manganese chloride	3*	66	32
Cobalt chloride	0·6*	60	31
Ferrous sulphate	2	59	23
Zinc acetate	2	57	27
Cadmium sulphate	0·6*	56	34
Barium chloride	3*	54	27
Calcium chloride	3*	53	18
Cadmium nitrate	0·6*	31	22
Ferrous ammonium sulphate	1	26	16
Lead nitrate	2*	22	20
Potassium chloride	3	19	11
Ferric chloride	0·6*	16	20
Sodium chloride	3	15	11
Cupric chloride	0·6*	4	20
Ammonium nitrate	8	−14‡	10
Ammonium sulphate	4	−15	10
Magnesium chloride	3	−30	14
Sodium nitrate	6	−88	1
Mercuric chloride	0·6*	−95	1

* Optimum concentration in a dilution series.
† Bold type indicates hatch as good as or better than in root diffusate, which is 100 ± 10.
‡ Negative sign indicates percentage inhibition compared with the hatch in water, which is 0 ± 10.

Because of its potency in causing *H. schachtii* eggs to hatch, we tested the ability of zinc chloride to hatch eggs of other *Heterodera* species: *H. avenae*, *H. carotae*, *H. cruciferae*, *H. glycines*, *H. goettingiana*, *H. rostochiensis*, *H. tabacum* and *H. trifolii*. With *H. avenae*, where the number of eggs which hatch in oat-root diffusate and in water is the same, zinc chloride inhibited hatching slightly. With *H. goettingiana*, where only very few eggs hatch either in water or in pea-root diffusate *in vitro*, a few more larvae hatched out in zinc chloride. With all the other species, zinc chloride caused more eggs to hatch than did water, and with most of these species hatching was as great as or greater than that in the root diffusate from their host plants. As with other agents that stimulate hatching, it is not known how Zn^{2+} and Cd^{2+} ions act.

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Failure of the Zona Reaction in Five Pig Eggs

In most mammals which have been examined, the first spermatozoon that penetrates into the egg stimulates the egg to undergo a reaction which prevents subsequent spermatozoa from traversing the zona pellucida[1]. This phenomenon is called the 'zona reaction'. In the pig, following the zona reaction, spermatozoa can still penetrate into the zona but they normally cannot traverse it[2]. However, eggs aged for several hours before exposure to the first spermatozoon sometimes fail to elicit the zona reaction and several spermatozoa enter the vitellus (polyspermy) to become male pronuclei (polyandry)[3].

In the course of examining several hundred recently fertilized pig eggs, we observed five eggs which did not exhibit a zona reaction. The first of these came from a litter of nine eggs recovered about 8 h after ovulation. Eight of these were at normal stages while one egg (egg *A*) was unusual in that it contained two spermatozoa in the perivitelline space (Fig. 1). After the egg was fixed in acetic-alcohol and stained with orcein, a vesicular nucleus was revealed (Fig. 2). Hancock[4] reported that out of 1,677 pig eggs examined, three had vesicular nuclei. The second egg that had not undergone a zona reaction came from a litter of five eggs recovered 54 h after insemination.

Four of these were normal, fertilized, four-celled eggs while the fifth (egg *B*) was one-celled and contained more than twenty spermatozoa in the perivitelline space (Fig. 3). Unfortunately, egg *B* was lost during fixation so that its nuclear status could not be determined. Eggs *C*, *D*, and *E* (Figs. 4, 5, and 6) were recovered 10–16 h after ovulation and were found to contain 10–15 accessory sperm in the perivitelline space and to have vesicular nuclei. We are not aware of any other recorded observation describing spermatozoa in the perivitelline space of the pig egg.

Pig eggs, and most other mammalian eggs examined, are normally in second metaphase at the time of ovulation. Polge and Dziuk[5] found twenty-eight eggs with vesicular nuclei during a study of 1,472 recently fertilized pig eggs, and nine of these twenty-eight each had a spermatozoon in the cytoplasm but pronuclei had not formed, such as is shown in Fig. 6. Some eggs, therefore, fail either to receive or to respond to the signal for initiation of meiotic development and maturation prior to ovulation. Eggs *A*, *C*, *D* and *E* had vesicular nuclei and therefore were not fully mature. This suggests that as an egg matures meiotically it concomitantly develops its ability to elicit a zona reaction. This hypothesis could be tested by transferring follicular eggs at various stages of maturation into the oviduct of recently mated sows. After an appropriate time-interval these eggs would be recovered and examined to see whether spermatozoa are present in the perivitelline space.

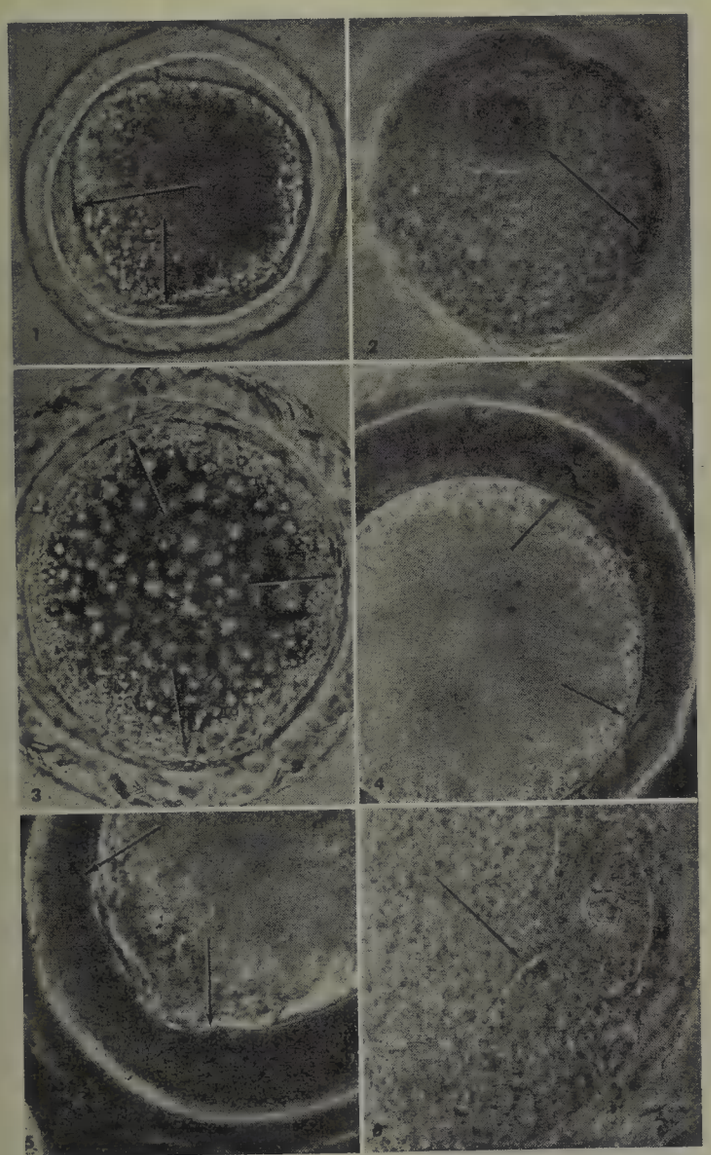

Fig. 1. Egg *A* with spermatozoa in perivitelline space (arrows) (fresh whole-mount; × *c.* 225)

Fig. 2. Egg *A* showing vesicular nucleus (arrow) (fixed and stained; × *c.* 125)

Fig. 3. Egg *B* showing several spermatozoa in perivitelline space (arrows) (fresh whole-mount, × *c*, 280)

Figs. 4 and 5. Eggs *C* and *D* showing spermatozoa in perivitelline space (arrows) (fresh whole-mounts, × *c.* 280)

Fig. 6. Egg *E* showing penetrated spermatozoon (arrow) near vesicular nucleus (fixed and stained, × *c.* 280)

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Effects of (2-Chloroethyl)trimethylammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees

The growth retardant *N*-dimethylaminosuccinamic acid (*B*-995) has been shown to modify growth in various fruit trees[1–3].

In experiments at East Malling, another growth retardant, (2-chloroethyl)trimethylammonium chloride (CCC), has shown similar effects on pear trees. A single spray with 1 per cent CCC, applied on May 25, 1964, to 1-year-old pear trees of the variety 'Williams' Bon Chrétien', halved the total new shoot-length and greatly increased the number of fruit buds. These effects were obtained regardless of any pre-treatment of the trees with gibberellic acid (GA).

Four sets of ten trees were sprayed with GA at 500 p.p.m. on the following dates: (*A*), April 18, April 29 and May 4, 1964; (*B*), April 29 and May 4; (*C*), April 29 only; (*D*), April 18 only. A fifth set (*E*) was left unsprayed. On May 25, half the trees in each set were sprayed with 1 per cent CCC (treatment *F*).

The results are shown in Figs. 1–3. The GA sprays had no effect on primary shoot growth, but the triple GA spray (treatment *A*) increased both secondary and axillary shoot growth. The single CCC spray dramatically reduced shoot growth, regardless of whether or not GA had previously been applied (Fig. 1). Fruit bud formation was reduced by triple GA spray, but greatly increased by the CCC spray in every case (Fig. 2). Fig. 3 shows the appearance of trees of the most contrasting treatments *E*, *EF*, and *A* in the following spring.

On April 26, 1965, when the trees were in full bloom two trees of each treatment were exposed to a freezing cycle,

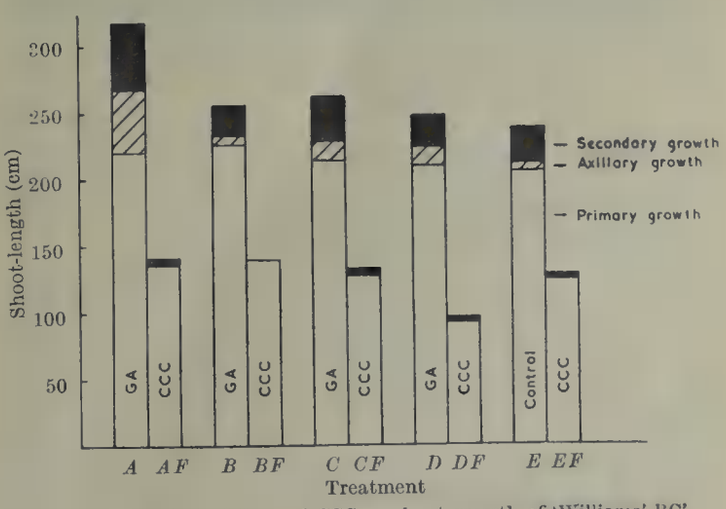

Fig. 1. Effect of GA and CCC on shoot growth of 'Williams' BC'

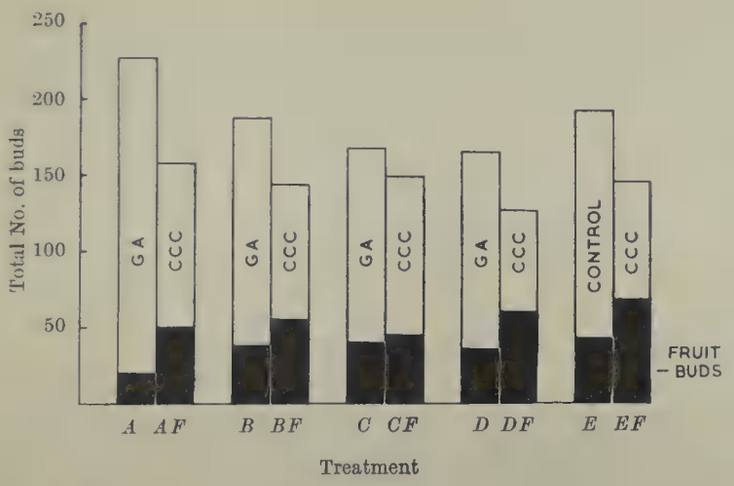

Fig. 2. Effect of GA and CCC on fruit bud formation of 'Williams' BC'

Fig. 3. 'Williams' Bon Chrétien' pear, a year after receiving treatments: *E*, unsprayed control; *EF*, 1 per cent CCC on May 25, 1964; *A*, GA 500 p.p.m. on 3 occasions. Photo, April 21, 1965

giving a temperature of $-3{\cdot}5°$ C for 15 min. The results (Table 1) showed that blossoms on the trees receiving a CCC spray in 1964 had a greatly increased frost resistance. The cell size of blossoms is being investigated.

Table 1. Effect of CCC Spray in May 1964 on Frost Damage to Flowers in 1965

Treatments	(Percentage flowers killed) *A*	*B*	*C*	*D*	*E*	Mean
Without CCC	80	85	93	54	94	81
With CCC	45	44	54	67	38	50

Growth retardants, used alone and in conjunction with gibberellins, open exciting new possibilities in modifying and controlling growth and fruitfulness of trees, and in the control of irregular bearing.

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Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in *Hedera*

The morphological change which accompanies a juvenile-to-mature transition in various plant species has received considerable attention in recent years[1-7]. Some workers have attempted to relate biochemical[8,9] and anatomical[10] factors to those changes which are so striking in certain plants as to give the impression that the mature phase is an entirely different species. These studies confirm the findings of Beakbane[11] and extend her hypothesis concerning adventitious rooting.

Leaf, stem, root, and vegetative bud tissue was taken from actively growing juvenile, transitional, and mature phase plants of ivy (*Hedera helix*) and prepared for microscopic examination. In addition, flower buds of mature plants were studied. Stem sections were taken 2 cm below the apex. All tissues were prepared by dehydrating in the tertiary butyl alcohol series, embedding in paraffin, staining with safranin-fast green, and sectioning with a rotary microtome at 10μ.

Tissues were examined for characters which might be associated with a change in growth phase. No gross anatomical differences were noted in the leaves, roots, or buds of juvenile and mature tissues. However, one striking difference was observed in the cross-section of the stem. In addition to the anticipated greater lignification of tissues in the mature stem, a discontinuous ring of fibres encircled the phloem in these plants (Fig. 1, top). Phloem fibres were extremely rare in juvenile stems (Fig. 1, bottom). Transitional stems, beginning to show some mature morphology, possessed a few fibres which became more numerous as the maturing plant progressed in age.

Previous reports of difficulty in the vegetative propagation of mature *Hedera*[9,12] resemble those reported by Beakbane[11] in various woody plants. She has shown that shoots of difficult-to-root plants often have a high degree of differentiation into fibres and sclereids. In 'Conference' pear an almost continuous ring of thick-walled fibres encircled the phloem. She found an inverse relationship between ease of rooting and continuity of the ring. Stoutemyer[10] found that the mature phase stem in apple contained more pericyclic fibres than the juvenile form. Differences in response of cuttings from non-flowering and flowering plants have been correlated with differences in anatomical structure, the flowering stem containing a higher proportion of xylem[13]. Flowering of seedlings was also found to be associated with the structure of the stem. Struckmeyer[14] showed that the cell walls of stems of various flowering plants had become greatly thickened, and changes in anatomical structure occurred early in the development of plants placed in an environment favourable to the formation of flower primordia.

This experiment supports Beakbane's suggestion that an increased difficulty in rooting is associated with increased fibre development in mature stems. Also supporting Beakbane, it is difficult to conceive that such a correlation could explain the rooting problem strictly on the basis of a mechanical barrier, since in no case observed in *Hedera* was the fibre ring continuous so as to form a lignified cylinder. Beakbane also refers to difficult-to-root plants, such as *Pittosporum*, in which no sclerenchyma is present. Recent experiments by Sachs, Loreti, and De Bie[15] showed no simple relationship between density and

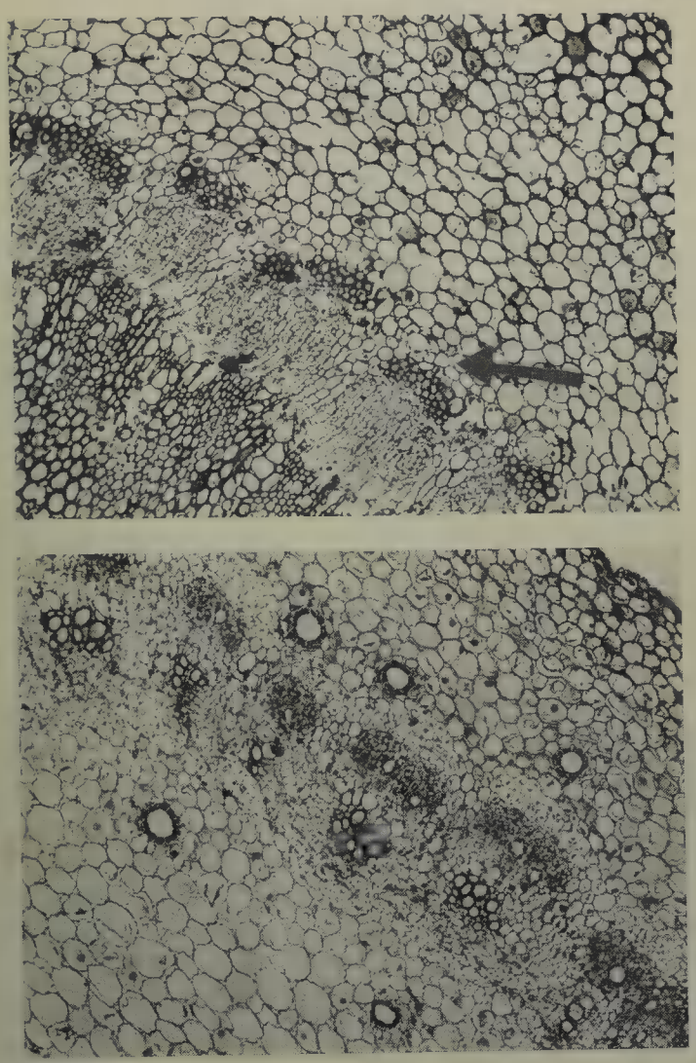

Fig. 1. Cross-sections of mature (upper) and juvenile (lower) stem of *Hedera helix*. Arrow points to fibres peripheral to the phloem in the mature stem

continuity of sclerenchyma and ease of rooting in seven varieties of olive cuttings. Indeed, if the correlation described by Beakbane, and supported by the present work, is significant, a chemical involvement must be assumed.

One other point deserves mention concerning *Hedera* morphology and anatomy. As the ivy proceeds from the juvenile to mature growth phase, changes in leaf shape, stem shape, anthocyanin content, formation of aerial roots, and phyllotaxy occur. In addition, growth habit changes from a distinct plagiotropic to an orthotropic form. Probably the strengthening of stems, and change to a shrubby habit, may be associated with the increased shoot lignification and development of fibres. The additional shoot strength imparted by the fibres is apparently sufficient to change *Hedera* from a vining to an upright growth habit.

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VIROLOGY

Milker's Nodule Virus Infections in Dorset and their Similarity to Orf

Between January 1964 and March 1965 seven farm workers on six Dorset farms developed lesions on the hand or forearm which were diagnosed as clinically typical orf infections. Material from each patient was examined by electron microscopy and tissue culture as previously described[1,2]. In each instance pox-virus particles were seen which in morphology and size were identical with orf virus[3]. In tissue culture four specimens produced the same cytopathic changes as orf virus and serial passage of three virus strains was obtained in primary human amnion and rhesus monkey kidney. No difference in behaviour was noted between these strains and orf virus strains previously grown from humans infected from sheep.

Orf virus infection of sheep is well known as contagious pustular dermatitis (CPD). None of our seven patients had been in contact with sheep, but all had milked cows within three weeks of onset of their lesions. Orf infection of cattle has not been reported, so these human infections would have been diagnosed as milker's nodules or pseudocowpox if the animal source of infection had been considered of prime importance[4–7].

Investigation of the six dairy herds revealed teat lesions on some animals in all six herds. Two distinct types of lesion are recognizable: (1) *Benign or chronic*. This is a mild erythema of the teat which is quickly followed by profuse scabbing. The scabs are soft, scurfy and yellowish-grey and are often rubbed off at milking. The teat surface becomes corrugated and prone to develop chaps and sores. The lesions are painless throughout their course and persist for several months. This type of lesion seldom affects many cows in a herd. (2) *Acute*. An erythematous eruption of the teat develops into a small vesicle or pustule which ruptures within 48 h. Prolific scab formation follows rapidly, some of the scabs being very large. Pain is only noticeable before scab formation, and indeed, scabs can be removed with no response by the cow. Granulation develops beneath the scab until the lesion becomes elevated some 2–3 mm above the general teat surface, and varies in diameter from 0·5 to 2·5 cm. The lesions heal from the centre, and, when the scab drops or is rubbed off, a characteristic horseshoe-shaped ring of minute scabs at the circumference is left. Each lesion takes 7–10 days to this stage. A small wart-like granuloma then remains, marked out in the early days by the horseshoe ring, and persists for many months, several of these making the teat very coarse. Spread of the lesions from cow to cow appears to be slow, and 5–10 per cent of the herd is usually affected at any one time. Freshly calved animals and those recently entering the herd are most often affected. Two herds had an epidemic of the infection which appeared a few weeks after their arrival from a different county.

Scabs were collected when present and small biopsies were made of the other types of lesion. As shown in Table 1, identical pox-virus particles were found in the

Table 1. Tissue Culture in Primary Human Amnion, Secondary Monkey Kidney and Primary Bovine Testis

Farm	Farm worker	Method of examination: Microscopy	Culture	Passages	No. of cattle examined	No. positive: Microscopy	Culture
1	R.T.	Positive	Positive	2+	3	3	3
2	S.T.	,,	,,	4+	0	.	.
3	M.J.P.	,,	,,	None	1	1	1
4	J.M.P.	,,	,,	3+	4	3	3
4	A.M.	,,	Negative	−			
5	Miss C.	,,	,,	−	4	4	N.D.
6	A.H.	,,	N.D.	.	6	4	N.D.

Passages given are number attempted. The virus from M.J.P. produced typical cytopathic changes but failed to passage. The others showed no evidence of decreasing infectivity when passage was discontinued.

N.D., not done.

five herds from which specimens were obtained and growth in tissue culture was successful. The virus behaved in every way like the human strains. Orf infection of lambs and ewes is enzootic in Dorset, the virus having been isolated by us both from sheep and from shepherds. The possibility of transfer of orf infection from sheep to cattle was considered, but no evidence of such transfer was found. In order to find out how common teat lesions might be among dairy herds in West Dorset which were not associated with human infections, 16 herds were inspected. Teat lesions were found in some animals in every herd. At least four presumptive human infections were also found although too late for laboratory investigation. Confirmation of the bovine infections was possible in 14 of the 16 herds. As only one specimen was examined from each of the two negative herds, this does not exclude their infection. Of 48 animals examined, 36 had the pox-virus infection, 5 bovine warts and 7 animals were negative. The infection is therefore enzootic. No marked seasonal variation was evident. Lesions of the mouth in calves have not been seen, but few calves were suckled for more than three days because these were dairy herds.

It has been found that electron microscopy is a quicker and more reliable method than tissue culture for examination of this kind of material. Bacterial and fungal contamination of the specimen is heavy and the virus grows slowly. When only small numbers of virus particles are present successful culture is far less likely, as shown in Table 2. No positive results were obtained with cultures when microscopy was negative. Microscopy is therefore an invaluable tool for this type of field investigation and could be applied on a large scale provided that adequate cultural confirmation is included.

Comparison of the growth of cattle and sheep virus strains in a variety of tissue culture cells is shown in Table 3. No consistent differences were observed in the range of cells affected, in the type of cytopathic change, or in the number of successful passages possible. Both viruses have a tendency to die out and the differences shown appear to be no greater than between different strains of either source. Growth in primary cells is more reliable and yields more virus than in continuous cell lines. Most strains have passaged 10 or more times in primary cells without dying out.

There are several aspects of the work presented that seem worth consideration.

Table 2. Comparison of Number of Bovine Poxvirus Particles seen by Microscopy with Cultural Result

Virus particles per grid square*	Tissue culture Positive	Negative
1,000–	1	0
100–1,000	10	0
10–100	8	6
1–10	6	4
0	0	12

* A standard 2·30 mm electron microscope grid has squares of approximately $100\mu \times 100\mu$.

(1) *Human lesions.* Infection with this bovine virus and ovine orf can produce a variety of clinical lesions in man from multiple vesicles to the classical single indurated nodule. When the animal source is unknown, or in doubt, it is not possible in our experience to differentiate between ovine and bovine infections. A wider experience might enable this, but all the dermatologists we have consulted so far have agreed that differential diagnosis is based on knowledge of the animal source. Thus shepherds are said to have orf whereas a similar lesion on a cowman is called a milker's nodule. We feel that a thorough comparison of the lesions is likely to show the same pathology irrespective of the source of infection.

(2) *Bovine lesions.* This infection of dairy cattle, hitherto unsuspected, is clearly enzootic in Dorset. That it came to our notice by the appearance of the human infections is suggestive of a higher recent incidence, perhaps due to changes in farming, as three infections were from two herds with epizootic infection in consequence of being recently moved into the county.

There is no information at present on the incidence of infection elsewhere in Britain, but it is probably widespread and might be the 'spurious cowpox' described by Jenner in 1799 (ref. 6).

Teat lesions in dairy cattle tend to be regarded as due to minor trauma, chapping in cold weather, etc. But it is worth consideration that even trivial lesions are prone to secondary bacterial infection and this is one way in which bacterial infection of a teat could occur which might lead to mastitis.

(3) *The relations of the bovine virus.* The poxviruses are divisible into two groups by morphology. The larger group have the vaccinia type of structure, the smaller one that described for orf[3,8]. We refer to the latter as the orf group because of the antiquity of the word 'orf', that is Old English *hreof*: rough, scabby[9], and orf-qualm: cattle plague[10], and the fact that this morphological type was first described by Abdussalam and Cosslett in 1957 (ref. 8) for ovine virus, in preference to the suggested use of 'paravaccinia' group recently proposed by Peters *et al.*[11].

So far the orf structural group includes the viruses of orf or contagious pustular dermatitis[3,8,12], milker's nodule or pseudocowpox virus[6,7], bovine papular stomatitis virus[13,14], chamois contagious ecthyma[15,16] and that described recently by Pournaki *et al.* as bovine *X* or *BX* virus[17]. We consider that our bovine virus is synonymous with those described as milker's nodule, pseudocowpox and *BX*. Its relationship to bovine papular stomatitis (BPS) virus remains to be determined. Plowright and Ferris[18] consider BPS to be synonymous with the proliferative stomatitis described in the United States by Olson and Palionis[19] and several other stomatitis agents. It is noteworthy that two of the American workers developed milker's nodes on their hands, and material from these injected into calves produced stoma-

Table 3. Growth in Tissue Culture

	Ovine strains				Bovine strains			
	Sheep	Human			Cattle		Human	
	CPD†	5957	25772	19510	24120	4390	21917	1637
Primary cell cultures								
Bovine testis	++	++	++	++	++	++	++	++
Human amnion	++	++	++	$+_4$	++	++	$+_2$	++
Monkey kidney	++	++	++	++	++	±	++	++
Cell lines								
HeLa strain 232	$+_4$	$+_{10}$	±	$+_4$	$+_6$	$+_2$	$+_3$	$+_3$
HeLa strain MK 2	±	±	±	±	±	++ (>7)	±	±
Mouse ascites tumour*	–	++ (>7)	±	–	$+_3$	–	$+_2$	–
Rabbit *RK* 13	–	±	±	±	±	±	±	±
KB	–	±	±	±	–	–	–	±
Mouse *L* cells	–	$+_2$	–	–	–	–	–	–
BHK 21 (hamster kidney)	–	±	–	–	–	–	–	–

++, Indefinite passage possible with cytopathic effect (CPE); $+n$, limited number of passages (last passage number with appreciable CPE indicated); ±, non-transmissible CPE from initial inoculum; –, no change.

* *In vitro* adapted Bandinelli strain.

† CPD is the Wellcome Laboratories vaccine strain.

titis. BPS strains are not readily available in Britain and we have been unable to find evidence of natural infections.

The relationship of the bovine strains to orf and the other animal viruses will remain conjectural until further work, especially serology, is complete, but on present evidence their similarity appears so close as to make separation difficult.

We thank Mr. G. H. C. Ellis, Mr. W. MacKinnon, Mr. J. Hall and Mr. M. H. Woodford for their help, and Prof. J. D. Boyd for enabling us to use the electron microscopy facilities of the Department of Anatomy, Cambridge.

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Plant Tumour Virus in Arthropod Host : Microcrystal Formation

The wound tumour virus (WTV) belongs to a group of plant pathogenic viruses that infect not only plants but also their respective arthropod carriers[1]. The virus has been detected in extracts of an insect vector and found to multiply in this vector, the clover leafhopper *Agallia constricta* van Duzee[2]. Purified preparations of WTV from plant and insect hosts permitted the determination of the particle size and its morphology[3]. Purified WTV particles, about 600 Å in diameter, have the shape of an icosahedron with a surface of 92 sub-units, about 75 Å in diameter[4]. The virus core stains heavily with uranyl acetate and comprises about 20 per cent of the volume of the particles. It consists of a double-stranded RNA[5]. The virus reaches considerable concentrations in extracts from insects and plant tumours, but the infection of the insect host is not apparent[3].

Until we undertook this investigation, it was not known whether WTV particles could be visualized in tissues of an insect vector *in situ*. The presence, in the cell cytoplasm of both healthy and virus-infected insects, of numerous spherical particles makes it extremely difficult to differentiate between normal cell constituents and virus, unless special circumstances arise which make identification possible.

Clover leafhoppers, *A. constricta*, were confined for 4 weeks to sweet clover (*Melilotus officinalis* L.) plants infected with WTV. Afterward, whole abdomens were dissected and fixed for 90 min in a 6 per cent glutaraldehyde and insect Ringer solution. This was followed by washing with 3 changes of distilled water for 30 min each, and further fixation in 2 per cent osmium tetroxide for 60 min. After dehydration in graded ethanol, the pieces were embedded in methacrylate or epoxy resin. Ultrathin sections were cut with a Porter-Blum *MT*–1 microtome, using a diamond knife for epoxy, and a glass knife for methacrylate resin. The sections, approximately 25–50 mμ thick, were double-stained (for epoxy embedded materials) with 6 per cent uranyl acetate for 60 min and with lead for 15 min[6]. Electron micrographs were taken with a Siemens Elmiskop *I*, with 80 kV accelerating voltage.

Although the size and shape of WTV were known from purified preparations, the identification of isolated particles would have presented a difficult task. Fortunately, striking accumulations of particles, forming crystalline arrays, were encountered in the cytoplasm of muscle and fat body cells. Since these particles, found in crystalline arrangement, resembled in size and shape WTV particles, and since such particles were never observed in non-infected insects, it was concluded that the regular accumulations were WTV microcrystals. This conclusion was further confirmed by the finding of particles of the same size and morphology in WTV-infected plants, particularly in root and stem tumours of diseased sweet clover plants, and in WTV enations of *Rumex acetosa* and enlarged veins of WTV-infected *Trifolium incarnatum*[7], but not in healthy plants of the same species. Similar regular arrangements and distances between particles of the same size as found in the microcrystals were observed by Whitcomb (unpublished results) in a cross-section of a pellet of purified WTV.

Fig. 1 shows one of the striking accumulations of WTV microcrystals in a single fat body cell of *A. constricta*. Individual microcrystals of compactly packed WTV particles were also encountered within microfibrils of muscle fibres, as shown in Figs. 2 and 3. These electron micrographs of microcrystalline virus inclusions indicate the regular, three-dimensional arrangement of the particles. When the microcrystals were found in fat body and muscle cells, it was not known whether they also occur in other organs of the arthropod host. The time-consuming part of our work was the subsequent identification of particles, particularly of individual ones or of very small WTV accumulations[8]. The finding of virus microcrystals, on the other hand, turned out to be easier than was anticipated. It should be pointed out that the size of the WTV inclusions is smaller than, or similar to, mitochondria, which makes their structural identification difficult under a light microscope.

The diameter of the virus particles within a microcrystal was calculated as 58–60 mμ. These measurements were made on epoxy embedded materials, including central darker areas surrounded by less dense regions.

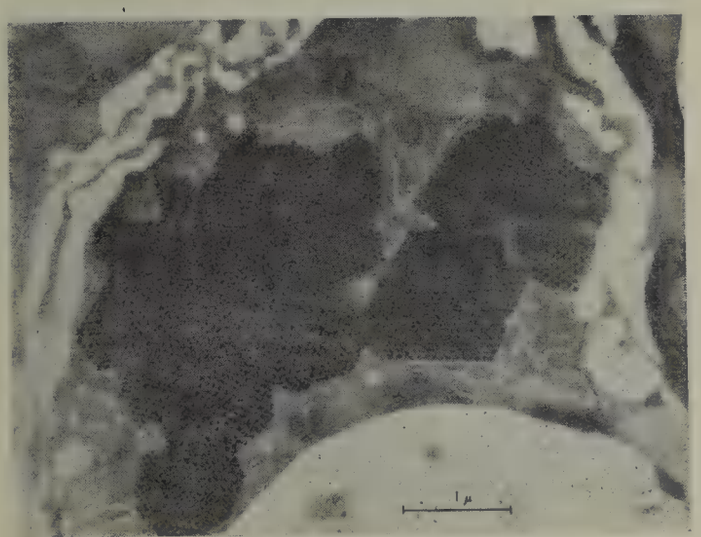

Fig. 1. Crystalline inclusions (microcrystals) of wound tumour virus (WTV) in the cytoplasm of a fat body cell of *A. constricta*

Parsons[9] summarized the differences in size between isolated virus particles and those obtained from ultrathin sections of animal viruses. It was known that methacrylate polymerization causes considerable shrinkage and distortion[10]. Therefore, the diameter of virus particles in methacrylate-embedded sections was expected to be smaller than the diameter of purified WTV particles. This was confirmed in the results shown in the legends to Figs. 2, 3 and 4. Centre-to-centre measurements resulted in different average diameters, when the measurements were made from different directions.

Electron micrographs of some WTV microcrystals clearly showed the regularly repeated pattern of darker and lighter particles (Fig. 4), corresponding with the denser and less dense masses of regularly arranged units. Obviously, in such sections, the cutting face of the knife was not parallel to the surface of the WTV microcrystal. The darker particles have a central core, while the less dark ones are devoid of the core.

The nature of the crystal formation is not clear. It is feasible to assume that they represent the result of virus multiplication at the site where they are found. In the case of animal viruses in animal host cells, such a hypothesis had been put forward by several workers[11,12]. However, some virus particles are not arranged regularly in the cytoplasm[13]. While it appears likely that virus multiplication occurred in the cytoplasm of the cells in which the microcrystals were encountered, these regular arrangements of particles could also represent the final stage of storage and accumulation of particles that have multiplied earlier in a viroplasmic matrix situated at another part of the cell.

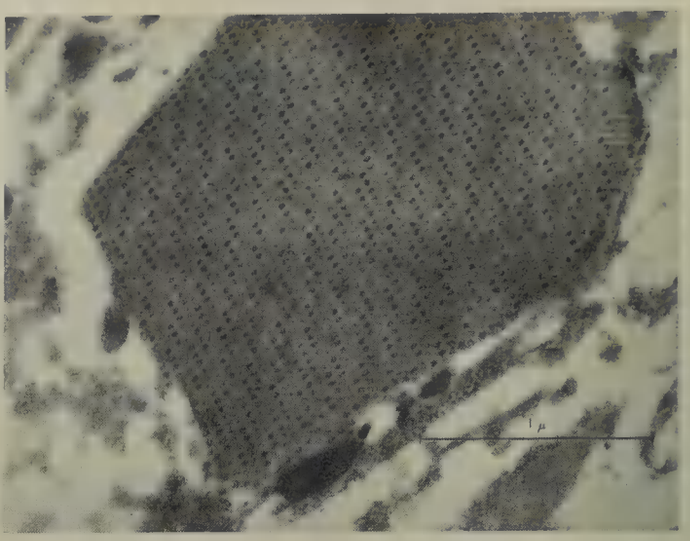

Fig. 4. Cross-section of a WTV microcrystal, embedded in methacrylate resin. Average diameters of 20 particles were: first direction—45 mμ; second direction—35 mμ. Average diameters of 10 particles in a loose cluster within same fat body cell were 57 mμ and 38 mμ for the two directions

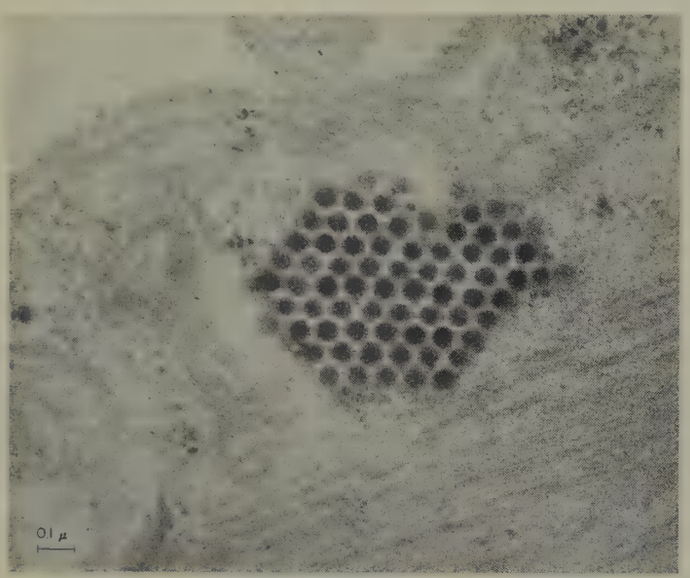

Fig. 2. Cross-section through a WTV microcrystal within microfibrils of a muscle cell of *A. constricta*. Epoxy resin embedding. Average diameters of 42 particles were: first direction (8 particles): 66·8 mμ; second direction (6 particles): 66·0 mμ; third direction (8 particles): 57·5 mμ; fourth direction (6 particles): 57·6 mμ; fifth direction (8 particles): 61·7 mμ; sixth direction (6 particles): 61·9 mμ. Single particles inside crystal (3 particles): 59·4 mμ single particle, outside microcrystal: 57·8 mμ

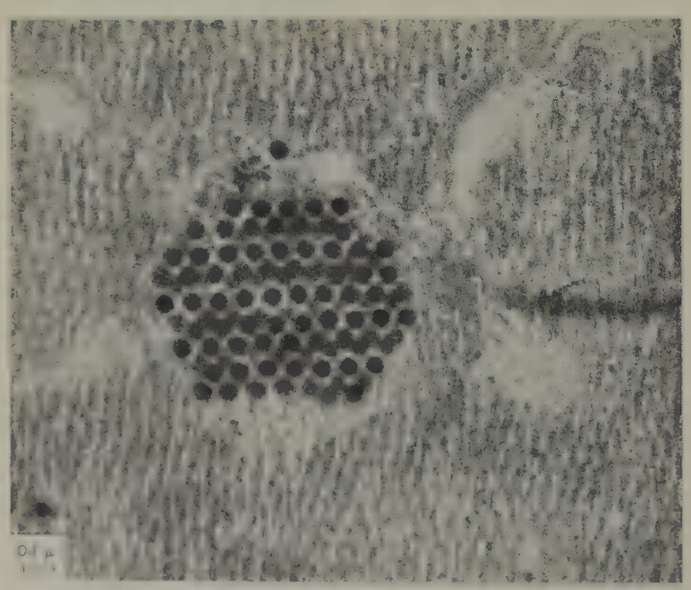

Fig. 3. Cross-section through a WTV microcrystal in *A. constricta* muscle cell. Epoxy resin embedding. Average diameters of 9 particles were: first direction—70·5 mμ; second direction—72·5 mμ; third direction—69·5 mμ. Single particle inside microcrystal measured 63·0 mμ × 59·6 mμ

The novel and unusual feature of the present findings is the formation of microcrystals of a plant-pathogenic virus in an animal host. So far as the authors are aware, this is the first such account. Regular arrangements of rice dwarf virus have also been reported in an insect vector[12], but electron micrographs of that virus did not suggest the formation of a crystalline three-dimensional structure. It should be pointed out that WTV microcrystals were also found in some cells of infected plants, alongside of large accumulations of virus particles and irregularly scattered WTV[7].

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VETERINARY SCIENCE

Development of *Eimeria tenella* in Avian Embryos

More than 300 species of the genus *Eimeria* have been recorded from birds and mammals[1]. Where details of the life-cycles are known, only two species appear to develop in sites other than the intestinal tract. These species, *E. stiedae* and *E. truncata*, occur in the liver of the rabbit and the kidney of the goose respectively and are not known to develop at other sites. Infection of hosts normally occurs after the introduction of sporulated oocysts by mouth and the development of species, other than *E. stiedae* and *E. truncata*, is confined to the intestine. *E. tenella* usually shows a rigid preference for the caecal mucosa of the domestic fowl although, in common with the other species of *Eimeria* in the fowl, the sporozoites are released in the small intestine. When this parasite is introduced by either the intravenous or intraperitoneal routes developing stages can only be located in the caeca[2–4].

This rigid site selection does not necessarily apply to *E. necatrix* or *E. brunetti* as sporozoites introduced directly into the caeca invade the walls and complete their life-cycles in the usual way[5,6]. The present investigation shows that *E. tenella* can develop in the chorioallantoic membrane of the chick embryo.

Sporozoites were released from the oocysts by using a modification of an *in vitro* excystation method[7]. In preliminary experiments, sporozoites injected into the allantoic cavity or intravenously caused the early deaths of ten-day-old embryos. Deaths usually occurred immediately after the intravenous injection of sporozoites whereas deaths occurred up to 24 h after allantoic injection and were associated with heavy bacterial contamination. To control contamination, the oocyst suspensions were incubated at 30° C for 24 h in peptone broth containing 50 I.U. penicillin and streptomycin per ml. This procedure was followed by a 1 h treatment in 1 : 500 'Chloros' solution and finally in 2 per cent formalin solution. The oocysts were washed in sterile 0·9 per cent sodium chloride between each treatment and then excysted. The findings following the inoculation of sporozoites are summarized in Table 1.

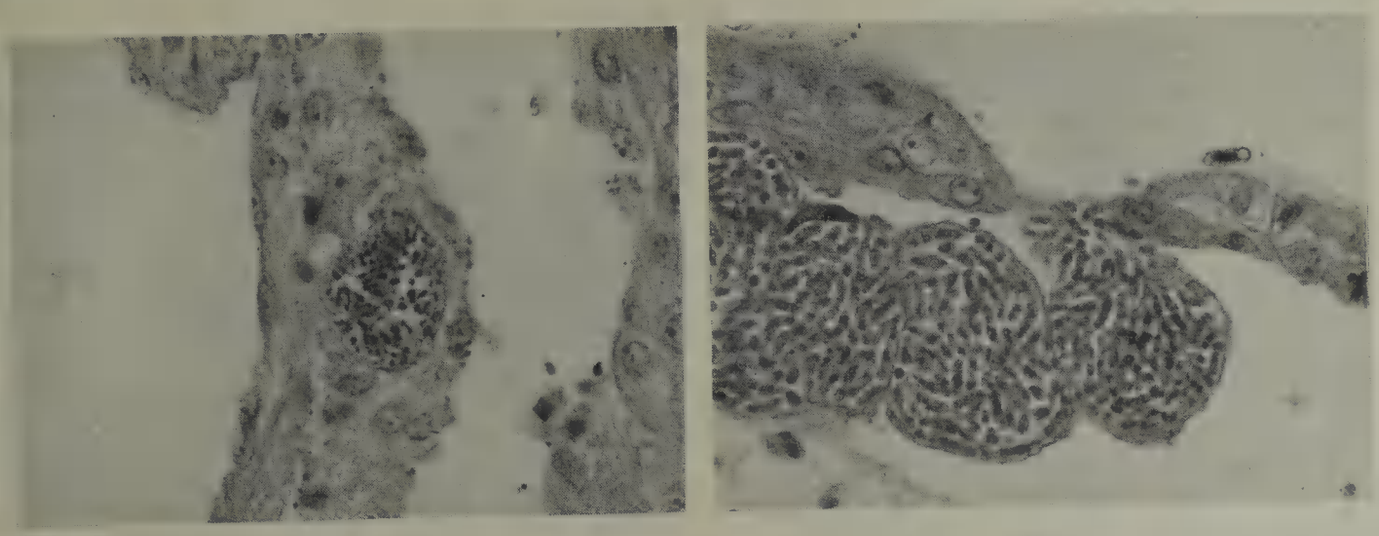
Sporozoites injected into the allantoic cavities of ten-day-old embryos produced infections and fewer early deaths than in six-day-old embryos. Intravenous inoculation of sporozoites produced no developing stages. Intra-amniotic inoculation was highly lethal and sudden deaths occurred associated with multiple haemorrhages.

In the infections which occurred from injections of sporozoites into the allantoic cavity parasites were only detected in the chorioallantoic membranes and not in the embryo itself. Immature and mature schizonts were seen in increasing numbers in histological sections of the chorioallantoic membranes on days 4, 5, 6 and 7 and numerous merozoites were found in the allantoic fluid on days 6 and 7. Schizonts, morphologically similar to those found previously, were also readily seen on days 8 and 9.

Table 1. The Effect of introducing Sporozoites of *E. tenella* into Avian Embryos by Different Routes

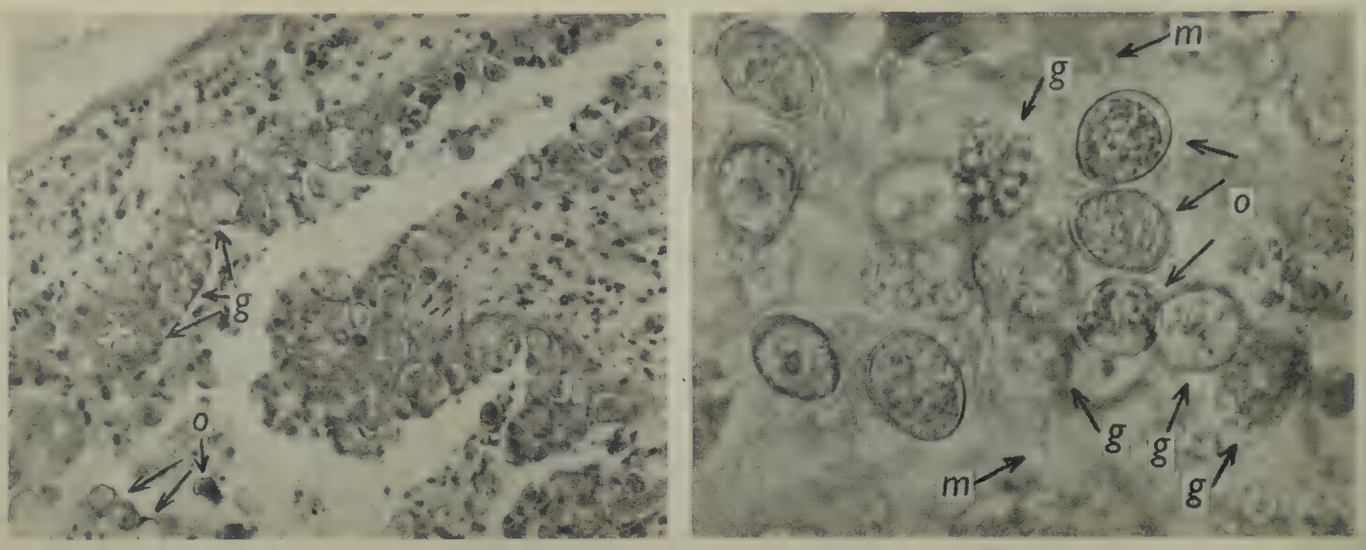
Number of sporozoites inoculated (10^3)	Volume inoculated (ml.)	Site of injection	Age of embryos	Number injected	Early deaths		Findings
					No.	Time	
140	0·1	Intravenous	11	6	5	Within 72 h	One survivor, killed day 7, no parasites
140	0·1	Allantois	11	6	3	Within 72 h	In 2 survivors schizonts and oocysts present in C.A.M.* on days 5 and 10 after infection. One survivor negative on day 10
24–38	0·05–0·08	Intravenous	5	9	2	2–24 h	All negative for parasites when examined between 5 and 11 days after inoculation
48–240	0·1–0·5	Amnion	10	8	8	Within 24 h	Multiple haemorrhages
108	0·1	Allantois	6	12	7	Within 48 h	In 3 survivors schizonts were found in the C.A.M. on days 4, 5 and 7 after inoculation. No parasites were found in embryos examined on days 8 and 9 after inoculation
64	0·05	Allantois	10	20	1	Within 24 h	The C.A.M.'s of all embryos were positive for parasites when examined between days 4 and 11 of infection. Schizonts were found from days 4–10, gametocytes and oocysts from days 7–11. Nine deaths occurred on day 7; numerous schizonts were seen in the C.A.M. and numerous free merozoites were seen in the allantoic fluid

* Chorioallantoic membrane.



(*a*) (*b*)

Fig. 1. Mature schizonts in chorioallantoic membranes; sections stained by haematoxylin and eosin (×600). (*a*) Schizont from 14-day-old embryo 4 days after inoculation: (*b*) schizonts from 16-day-old embryo 6 days after inoculation



(*a*) (*b*)

Fig. 2. (*a*) Gametocytes (*g*) in epithelial cells of the chorioallantoic membrane and oocysts (*o*) free in the allantois of a 20-day-old embryo, 10 days after inoculation with sporozoites. Stained by haematoxylin and eosin (× 210). (*b*) Oocysts (*o*), gametocytes (*g*) and merozoites (*m*) in the same embryo. Unstained preparation (× 600)

These schizonts were similar to the second generation schizonts of *E. tenella* in the caeca of hatched chickens on days 5 and 6 after receiving sporulated oocysts *per os*. The nine embryos found dead on day 7 after inoculation were presumed to be due to the very large numbers of schizonts in the chorioallantoic membrane and haemorrhage which occurred in the allantoic cavities. Gametocytes and oocysts were found in small numbers on day 7 and were found in increasing numbers until day 11. Oocysts were found free in the allantoic fluid and in necrotic material sloughed off from the chorioallantoic membranes. Photomicrographs of the different stages observed are shown in Figs. 1 and 2. The oocysts recovered from a ten-day-old infection sporulated normally and produced typical caecal infections when inoculated into one-week-old chickens.

The results show that the life-cycle of *Eimeria tenella* can occur at a site other than the caeca of the chicken and this may have important consequences. The life-cycle appears to be slightly delayed (large numbers of oocysts occurring after day 9 of the infection) and the phase of schizogony extended.

Maintenance of the avian *Eimeria* normally necessitates frequent passaging in fowls kept in strict isolation. The growth of *E. tenella* in the chick embryo may provide a more convenient and less expensive alternative. It is possible that other species of *Eimeria* also develop in embryos. An extension of this work is proceeding with other species of *Eimeria* of the fowl and *E. stiedae* of the rabbit. The method may be of use in the evaluation of anti-parasitic substances and, in addition, provides bacteria-free material for immunological investigations. Of particular interest is the apparent prolongation of schizogony of the parasite and the opportunity now afforded for studying the parasite in a host in which resistance is minimal[8].

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MISCELLANEOUS

Effect of Certain Types of Paper on Sexual Maturation of the Insect *Pyrrhocoris apterus*

On reading the article entitled "Juvenile Hormone Activity for the Bug, *Pyrrhocoris apterus*", by Sláma and Williams[1], I was struck by a likely correlation.

These authors report that this European insect fails to undergo normal sexual maturation in the presence of certain American papers (*New York Times*, *Wall Street Journal*, *Boston Globe*, *Science* and *Scientific American*), while British papers (*The Times* and *Nature*) had no inhibitory effect on the attainment of normal sexual maturity by this insect.

The active principle in the paper was found to be heat stable (100° C) and insoluble in water, but soluble in methanol, acetone, ether and petroleum ether. The authors ascertained that balsam fir, hemlock and yew contained the active principle, while red spruce, European larch and southern pine possessed very little activity.

The heart wood of Douglas fir contains dihydroquercetin as was first shown by Pew[2], and this is well known in the American wood pulping industry, for this substance interferes with pulping and tends to oxidize to quercetin, which is yellow on account of its cinnamoyl resonance; this spoils the appearance of the paper, so steps are taken to prevent its oxidation.

Moewus[3] showed that quercetin occupies a central position in the development of sexuality in the primitive biflagellate alga *Chlamydomonas eugametos*, being a precursor of isorhamnetin required in the female gametes and of peonin required in the males.

It might therefore be profitable for entomologists to ascertain whether the inhibitory substance may not be dihydroquercetin, which could well interfere with sexual maturation if quercetin should be a step in the pathway to sexual maturity in these insects, as it is in *Chlamydomonas eugametos*.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 1

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 9.30 a.m. and 2.15 p.m.—Colloquium on "HS 303 (Early Bird) and the Post Office Earth Station at Goonhilly".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. R. Reid: "Ageing".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. D. A. Price Evans: "Genetics and Drug Idiosyncrasy".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. I. Scrub-itch and the Ecologist".*

UNIVERSITY OF LONDON (in the Chemistry Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. J. Millen: "Molecular Measurements" (Inaugural Lecture).*

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "The Doctor's View of Engineering: The Engineer's View of Medicine".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Island" (film with introduction by Prof. C. A. Fisher).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dame Kathleen Lonsdale, D.B.E., F.R.S.: "Crystallography as a Research Tool in Chemistry" (Jubilee Memorial Lecture).

Tuesday, November 2

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Guides to Action: Is There a Universal Teaching of Mankind ?"*

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, London, W.12), at 3 p.m.—Prof. P. Polani: "Human Chromosomes and their Abnormalities (II)".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. A. Banks: Presidential Address.

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. H. D. Anthony: "The Study of Scenery".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. B. Kinmonth: "Angiology of the Human Lymph System". (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in the Large Physics Lecture Theatre, Westfield College, Kidderpore Avenue, London, N.W.3), at 5.30 p.m.—Dr. J. Sicher (Czechoslovak Academy of Science): "The Steric Structure of Medium Ring Compounds and Approaches to Conformational Analysis".*

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Fundamental Ideas". (First of four lectures on "Magneto-Fluid Dynamics".)*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. H. Birtles: "The Government Services for Exporters".

Tuesday, November 2—Wednesday, November 3

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Surges in Pipelines".

PLASTICS INSTITUTE (at the Connaught Rooms, Great Queen Street, London, W.C.2)—Conference on "Automatic Control for Plastics Processing".

Wednesday, November 3

BIOMETRIC SOCIETY—BRITISH REGION (at the Wellcome Building, Euston Road, London, N.W.1), at 2.30 p.m.—Symposium on "Non-Parametric Methods". Principal Speakers: Mr. P. Sprent, Prof. P. Armitage, Mr. I. D. Hill and Dr. G. B. Wetherill.

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 3 p.m.—Mr. A. C. Hardy: "The Colour Coordination of Tiles and other Factory Coloured Products for the Building Industry in relation to BS 2660"; Mr. F. Malkin: "An Experiment in the Colour Tolerance of Glazed Wall Tiles".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. A. Bradley and Mr. C. Clarke: "Atmospheric Radio Noise and Signals Received on Directional Aerials at High Frequencies"; Mr. C. Clarke, Mr. P. A. Bradley and Mr. D. E. Mortimer: "Characteristics in Atmospheric Radio Noise Observed at Singapore".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Distribution and Application of Electricity in Building Sites".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Mr. M. H. Freeman: "Long Range Weather Forecasting".

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. R. W. Horne: "Electron Microscopy on the Macromolecular Level".

SOCIETY OF NON-DESTRUCTIVE EXAMINATION (in the Weir Lecture Hall, Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, W.1), at 5.30 p.m.—Mr. B. R. Byrne: "The Selection, Training and Briefing of N.D.T. Personnel" (Third Croxson Memorial Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. II. Akamushi: The Red Mites of Japan".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. B. L. Clarkson: "High Intensity Acoustic Noise Sources".

Thursday, November 4

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "Advanced Methods of Energy Conversion—Magnetohydrodynamic Power Generation" organized by Dr. L. Rotherham, F.R.S.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at the School of Pharmacy, 29 Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "Recent Developments in Microbiology in the Beverage Industries".

UNIVERSITY OF LONDON (at King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 4.30 p.m.—Prof. H. Rahn (U.S.A.): "The Physiology of the Diving Women of Korea and Japan".*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Institution of Electronic and Radio Engineers, at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Selection of Topics for Research in Electrical and Electronic Engineering" opened by Sir Robert Cockburn, K.B.E., Dr. E. Eastwood, C.B.E., Prof. Sir Willis Jackson, F.R.S., and Dr. L. Rotherham, F.R.S.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. C. Phillips: "The Structure of Lysozyme". (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in the Large Physics Lecture Theatre, Westfield College, Kidderpore Avenue, London, N.W.3), at 5.30 p.m.—Dr. J. Sicher (Czechoslovak Academy of Science): "Conformational Equilibria in Disubstituted Cyclohexanes".*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Dr. H. N. Daglish: "Applications of Low Temperatures in Satellite Communications".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Relative Merits of Once-Through Versus Other Types of Boiler Circulation".

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Steady Flow". (Second of four lectures on "Magneto-Fluid Dynamics")*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brunel College Chemical Society, at Brunel College, Woodlands Avenue, London, W.3), at 7 p.m.—Mr. M. C. Hyde: "Collecting Information on the Chemical Industry".

Friday, November 5

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. W. Cannon, Mr. G. C. Rider and Mr. D. Wilkinson: "Operational Experience with Tropospheric Scatter Systems".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Fine Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. Gerrard: "Studies in Boron Chemistry: Organic Analogues of Heterocyclics".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. D. C. Phillips: "The Structure and Function of Lysozyme".

Saturday, November 6

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Michael Boorer: "Rhinoceroses".*

Monday, November 8

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. R. A. Horsley: "Creep Properties of Thermoplastics".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. G. P. C. Chambers: "The Colloidal and Surface Properties of Clay Minerals".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Hatfield College of Technology, Hatfield), at 6 p.m.—Discussion on "Is Laboratory Work Really Necessary ?" opened by Dr. K. R. Sturley.

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fuel Element Behaviour in Gas-Cooled Reactors".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. A. G. Page: "Some Manufacturing and Processing Techniques in the Electronics Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Lord Rennell of Rodd: "Heinrich Barth and the Opening Up of Central Africa".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT or RESEARCH FELLOW (graduate in physics or engineering) IN THE DEPARTMENT OF CIVIL ENGINEERING, to work on problems of creep in concrete—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 5).

Research Assistant (with a degree in mathematics, physics or chemistry and preferably some postgraduate experience) in the Chemistry Department, to help in the devising and running of computer programmes for large problems in crystallography and theoretical chemistry under the supervision of Prof. D. W. J. Cruickshank—The Secretary of the University Court, University of Glasgow, Glasgow, W.2 (November 5).

Assistant Librarian (university graduate and chartered librarian)—The Principal, Lanchester College of Technology, Priory Street, Coventry (November 8).

Graduate in the Department of Botany for work on the physiology of insect-pathogenic fungi—The Registrar, University Senate House, Bristol, 2 (November 8).

Research Assistant (preferably with a degree in engineering) in the Department of Mechanical Engineering in the Faculty of Science, to operate research equipment engaged in investigation of combustion in reciprocating engines, to help in analysis of observations, and to assist in the development of instrumentation—The Registrar, The University, Manchester, 13, quoting Ref. 185/65 (November 10).

Lecturer (graduate with some years of teaching experience at degree level, and preferably an interest in control systems work or in computing) to assist in the Faculty of Technology—The Academic Registrar, Enfield College of Technology, Enfield, Middlesex (November 12).

Special Terminable Assistantship in Applied Psychology (full-time post) with duties in either industrial or educational psychology—The Secretary, University College, Cork, Republic of Ireland (November 12).

Superintendent (graduate in physics, preferably with experience of administration in industry, in the government service or in universities) of Laboratories in the Department of Physics—The Registrar, University College of Swansea, Singleton Park, Swansea (November 12).

Lecturer in Medical Physics—The Registrar, The University, Manchester, 13, quoting Ref. 188/65 (November 13).

Reader in Statistics at the London School of Economics—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 18).

Lecturers/Assistant Lecturers (3) in the Department of Pure Mathematics—The Registrar, University College of Wales, Aberystwyth (November 19).

Chair of Pure Mathematics—The Registrar, University of Newcastle upon Tyne, 6, Kensington Terrace, Newcastle upon Tyne 2 (November 20).

Lecturer in Philosophy—The Secretary of the University Court, University of Glasgow, Glasgow, W.2 (November 20).

Lecturer in Social Anthropology—The Secretary, The Queen's University, Belfast, Northern Ireland (November 20).

Reader, Senior Lecturer, Lecturer or Assistant Lecturer in Physics in the School of Mathematics and Physics—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (November 20).

Chair of Organic Chemistry at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 22).

Chair of Microbiology in the Faculty of Medicine—The Secretary, The Queen's University, Belfast, Northern Ireland (November 24).

Chair of Biochemistry—The Secretary of the University Court, The University, Glasgow (November 26).

Chair of Physiology—The Secretary of the University Court, The University, Glasgow (November 26).

Chair of Social and Preventive Medicine at the University of the West Indies—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (November 29).

Lecturer (preferably with special interests and qualifications in terrestrial zoology with emphasis on entomology, especially the ecology of insects) in Zoology at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

Lecturer (with a distinguished academic record and preferably with a research interest in one of the following fields: vertebrate physiology, embryology or tissue culture, cell biology, invertebrate zoology, other than entomology or marine biology) in the Department of Zoology, School of Biological Sciences, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

Lecturers (2) (one preferably theoretical reactor physicist with experience in modern computational techniques, and the other well qualified in the fields of fluid dynamics and heat transfer) in the Department of Nuclear Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (November 30).

Lecturer (Grade II) in the Department of Sociology—The Registrar, University Senate House, Bristol, 2 (December 1).

Temporary Lecturer in Zoology at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 4).

Senior Fellow or Fellow; and a Senior Research Fellow or Research Fellow in the Department of Mathematics, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 6).

Senior Lecturer/Lecturer in the School of Textile Technology, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 12).

Entomologist (national of the United Kingdom or the Republic of Ireland, with a B.Sc. degree and preferably experience of African conditions) in Bechuanaland, to undertake a survey of the cotton, maize and sorghum pests and make recommendations as to their control—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. 213/19/06.

Head of the Mathematics Department—The Master, Dulwich College, London, S.E.21.

Lecturer (with a good honours degree in zoology and preferably a higher degree)—The Registrar, Regional College of Technology, Leicester.

Research Assistant in the Department of Agricultural Botany for work on soil microbiology—The Registrar, University College of Wales, Aberystwyth.

Technician/Senior Technician in the Department of Biological Sciences—The Secretary, Bradford Institute of Technology, Bradford 7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Royal Institute of Chemistry. Lecture Series 1964. No. 3: Orbital Degeneracy and Spin Resonance of Free-Radical Ions. By Dr. A. Carrington. (Meldola Medal Lecture 1964.) P. 22. 4*s*. No. 4: Relaxation Methods for Studying Very Rapid Reactions in Solution. By Dr. D. N. Hague. Pp. 16 4*s*. No. 5: The Chemistry of Carbenes. By Dr. A. Ledwith. Pp. 66. 6*s*. (London: The Royal Institute of Chemistry.) [149

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 58: Sine-Squared Pulse and Bar Testing in Colour Television. Part 1: Pulse and Bar Testing of the Chrominance Channel. Part 2: An Augmented Pulse and Bar Waveform for Testing the Complete Colour Signal. By L. E. Weaver. Pp. 27. (London: British Broadcasting Corporation, 1965.) 5*s*. [149

The Royal Observatory, Edinburgh. Publications—Vol. 3, No. 9: The Cygnus II Association. I. Intercomparison of Photometrics with the Edinburgh and Hamburg Schmidt Telescopes. By L. C. Lawrence and V. C. Reddish. Pp. 279–309. (Edinburgh and London: H.M. Stationery Office, 1965.) 9*s*. net. [149

London and Home Counties Regional Advisory Council for Technological Education. Engineering Education in the Region. Ninth edition. Pp. 48. (London: London and Home Counties Regional Advisory Council for Technological Education, 1965.) 5*s*. [149

Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964–65—Recruitment to the Civil Service. Pp. xxxviii+269. (London: H.M. Stationery Office, 1965.) 26*s*. net. [149

Library Association Pamphlet No. 24: Central Library Storage of Books. By P. W. Plumb. Pp. 57. (London: Library Association, 1965.) 16*s*.; (L.A. members 12*s*.) [149

Standing Committee on National Parks. Study No. 1: Afforestation in National Parks—a Policy for Tree Planting and Management in National Parks. Pp. 13. 1*s*. Study No. 2: The Future of National Parks and the Countryside. Pp. 17. 1*s*. (London: Standing Committee on National Parks of the Council for the Preservation of Rural England and the Protection of Rural Wales, 1965.) [149

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1133–G: Geology and Hydrology of the Hartford Research Center, CANEL Site, Middletown, Connecticut. By J. A. Baker, S. M. Lang and M. P. Thomas. Pp. iv+42+plates 1 and 2. Bulletin 1199–F: Bauxite Deposits of the Springvale District, Georgia. By Lorin D. Clark. Pp. iii+24+plate 1. Water-Supply Paper 1578: Geology and Ground-Water Resources of the Bristol-Plainville-Southington Area, Connecticut. By A. M. La Sala, Jr. Pp. iv+70+plates 1–3. Water-Supply Paper 1615–G: Testing Procedures and Results of Studies of Artificial Recharge in the Grand Prairie Region, Arkansas. By R. T. Sniegocki, F. H. Bayler, Kyle Engler, and J. W. Stephens. Pp. iv+56. 25 cents. Water-Supply Paper 1649: Water for Oregon. By K. N. Phillips, R. C. Newcomb, H. A. Swenson, and L. B. Laird. Pp. x+150. 60 cents. Water-Supply Paper 1772: Geology and Ground-Water Resources of Prowers County, Colorado. By Paul T. Voegeli, Sr., and Lloyd A. Hershey. Pp. vi+101+plates 1–6. Water-Supply Paper 1788: Ground-Water Resources and Geology of Niobrara County, Wyoming. By Harold A. Whitcomb. With a section on Chemical Quality of the Ground Water by T. Ray Cummings. Pp. v+101+plates 1–3. (Washington, D.C.: Government Printing Office, 1965.) [149

United States Department of the Interior: Geological Survey. Water-Supply Paper 1790–B: Summary of Floods in the United States During 1960. By J. O. Rostvedt. Pp. vi+147. 45 cents. Water-Supply Paper 1809–C: Ground Water in the Upper Star Valley, Wyoming. By Eugene H. Walker. Pp. iv+27+plate 1. 15 cents. Water-Supply Paper 1809–G: Ground-Water Resources on North Park and Middle Park, Colorado—a Reconnaissance. By Paul. T. Voegeli, Sr. Pp. iv+54+plates 1 and 2. Professional Paper 491–A: Introduction, Spread and Area Extent of Saltcedar (*Tamarix*) in the Western States. By T. W. Robinson. Pp. iii+12+plate 1. Professional Paper 498–B: Variations in Chemical Character of Water in the Englishtown Formation, New Jersey. By Paul R. Seaber. Pp. iii+35. 30 cents. (Washington, D.C.: Government Printing Office, 1965.) [149

The Law in Nature and the Dynamics of Openness. By Ernest L. Remits. Pp. iv+96. (Ottawa: The Runge Press, Ltd., 1965.) [410

World Health Organization. Protection of the Public in the Event of Radiation Accidents. (Proceedings of a Seminar jointly sponsored by the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, and the World Health Organization, Geneva, 18–22 November 1963.) Pp. 370. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 22 Sw. francs; 37*s*. 6*d*.; 7·25 dollars. [410

Annals of the New York Academy of Sciences. Vol. 126, Article 1: Viral Diseases of Poikilothermic Vertebrates. By M. H. Attleberger and 71 other authors. Pp. 1–680. (New York: New York Academy of Sciences, 1965.) 12 dollars. [410

Proceedings of the Preliminary Meeting on College Level Mathematics Education, Katada, 1964. (Under the auspices of the U.S.–Japan Program on Scientific Co-operation.) Pp. xi+96. (Tokyo: Japan Society for the Promotion of Science, 1965.) [410

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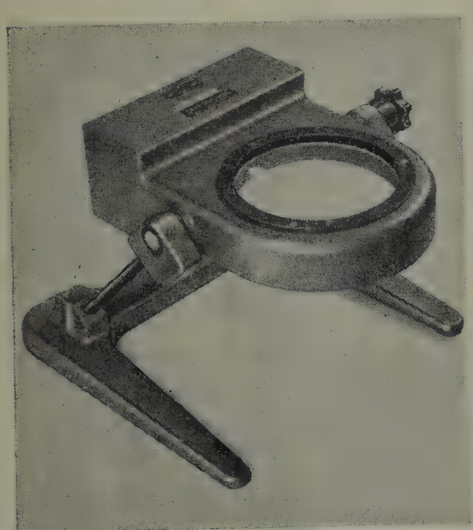

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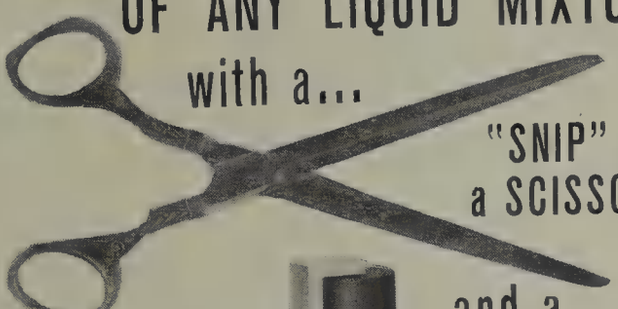

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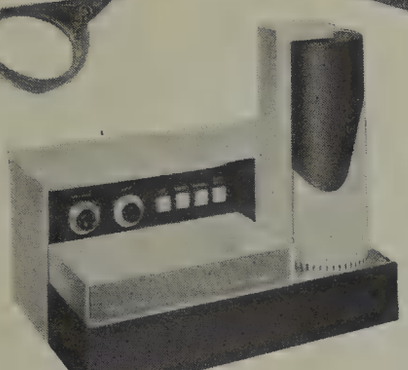

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APPOINTMENTS VACANT

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Applications are invited from graduates in Mathematics, Physics or Engineering for appointment as Computer Assistant in the Department of Mechanical Engineering on a research project concerned with heat and mass transfer through turbulent boundary layers. The work will involve the solution by numerical analysis of boundary layer equations of momentum and energy and correlation of experimental results. Previous experience in computing would be an advantage. The appointment is for a period of three years. Commencing salary according to qualifications and experience in the scale £920 by £30 to £1,010 per annum.

Application forms are obtainable from the Registrar of the College, Sackville Street, Manchester 1, to whom completed forms should be returned by November 13, 1965.

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Applications are invited for a LECTURESHIP or ASSISTANT LECTURESHIP in MATHEMATICS in QUEEN'S COLLEGE, DUNDEE, with effect from January 1, 1966, or ■ soon thereafter as may be arranged. Preference for appointment will be given to those with special interests in numerical analysis, but applications from those with other interests in pure or applied mathematics are invited. The department is undergoing expansion and there are excellent opportunities for both teaching and research in pure and applied mathematics. There is a Stantec "Zebra" digital computer in the College which is available for teaching and application of numerical techniques; the College hopes to acquire a modern and much larger computer early in 1967.

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Applications (6 copies), containing the names of three referees, to be lodged by November 20, 1965, with the Secretary of the University, Queen's College, Dundee, from whom further particulars may be obtained.

BEDFORD COLLEGE
(UNIVERSITY OF LONDON)

Applications are invited for the post of Research Assistant to Professor Monica M. Cole, Professor of Geography, on a special research project sponsored by the Natural Environment Research Council concerned with the distribution and origin of soils in the Southern Pennines. Applicants should be graduates preferably with some research experience and be prepared to work away from London. Appointment for three years in the first instance. Salary either in the range £700 to £850 or £925 to £1,050 according to qualifications and experience.

Applications (three copies) should reach the Secretary, Bedford College (University of London), Regent's Park, London, N.W.1, not later than Monday, November 8, 1965.

Scientific Civil Service

ELECTRICAL ENGINEERING/ELECTRONICS

The following are examples of existing vacancies, which may be filled at SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER level.

RESEARCH STATION, P.O. ENGINEERING DEPARTMENT, Dollis Hill, N.W.2.: Electrical Engineer (or Physicist), preferably with a mathematical bias, to work on CHARACTER RECOGNITION applied to MAIL SORTING. Formulation of decision logic and evaluation of partial and complete systems by computer simulation involved. Close liaison with design team.

ROYAL RADAR ESTABLISHMENT, Malvern, Worcs.: Electronics/Electrical Engineer for research on MICROMINIATURISATION techniques and their applications to various equipment problems. Studies of reliability and the use of redundancy in electronic equipment design.

NATIONAL PHYSICAL LABORATORY, Teddington, Middlesex: Electronics Engineer (or Physicist) to work in collaboration with the Physicists studying GAMMA RAY DOSIMETRY and measurement of neutrons and of radioactivity. To be responsible for advanced circuit design, applying semiconductor techniques and devices to problems of RAPID PARTICLE COUNTING, data handling and processing and very low current measurement. Some familiarity with computer logical design advantageous.

MARINE LABORATORY, DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND, Aberdeen: Electrical Engineer (or Physicist) for duties including the development of INSTRUMENTS AND TECHNIQUES FOR OCEANOGRAPHY AND FISHERIES RESEARCH. Duties at sea involved. Knowledge of acoustics advantageous.

PROOF AND EXPERIMENTAL ESTABLISHMENT, Shoeburyness, Essex: Electronics/Electrical Engineer for work on development of electronic and optical INSTRUMENTATION FOR BALLISTIC MEASUREMENTS and the subsequent automatic handling of data. Relevant experience, particularly of digital devices, necessary.

There are many other vacancies in most scientific disciplines. Recruitment by the Civil Service Commissioners is to permanent and pensionable posts. APPLICATION FORMS from Civil Service Commission, Savile Row, London, W.1, stating age and qualifications. (Reference: S/52-53).

DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

FRESHWATER FISHERIES LABORATORY
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A free booklet entitled 'THE SCIENTIFIC CIVIL SERVICE' is available on request from Civil Service Commission, Savile Row, London, W.1.

HEINZ 57 SCIENTISTS FOR RESEARCH AND DEVELOPMENT

Heinz need scientific staff for research into food processing and packaging at their new research centre recently opened in pleasant parkland at Hayes, Middlesex. Laboratory and pilot shop facilities are excellent. However, research and investigations cannot be carried out with modern equipment alone. They need more well-qualified scientific staff, specifically:

BIOCHEMISTS • CHEMICAL ENGINEERS CHEMISTS • FOOD SCIENTISTS PHYSICISTS • PHYSICAL CHEMISTS MICROBIOLOGISTS

Work covers most technological aspects of food processing and, in particular, the Physicist/Physical Chemist will be engaged on investigations into technological aspects of food packaging. Applicants should have at least a good honours degree or appropriate Diploma of Technology. Research experience or Ph. D. would be an asset for the Biochemical post. The upper age limit in all categories is 32, although this is by no means inflexible. Salaries and working conditions are those of a large progressive company. Financial assistance in changing location can be granted in certain cases.

Apply in writing, giving a brief personal history, to:

The Personnel Officer,

H. J. HEINZ COMPANY LTD.

Hayes Park, Hayes, Middlesex

ABBOTT LABORATORIES LIMITED

have a vacancy for a

DEPUTY MANAGER—QUALITY CONTROL

Applicants, aged 27 or over, should have, as a minimum, a University honours degree in Chemistry or an equivalent qualification and have spent several years working in a Quality Control Department, preferably in the Pharmaceutical Industry.

After a period of 1–2 years, promotion to Quality Control Manager is expected.

Commencing salary will be agreed by negotiation. The company has an attractive Pension Scheme with free life insurance and other benefits.

Applications, stating age and giving details of qualifications and previous experience, should be addressed to :

The Personnel Manager,
ABBOTT LABORATORIES LIMITED,
Queenborough, Kent, Marking the application N7.

UNIVERSITY OF CAMBRIDGE

Applications are invited for the post of Graduate Research Assistant in the Department of Radiotherapeutics. Candidates should have research experience in organic chemistry and radioactive techniques, and preference will be given to applicants holding a doctor's degree. The post will be for one year in the first instance with the possibility of extension for two or more years. The salary will depend upon previous experience with a maximum of £1.500 per annum. It is possible that the F.S.S.U. facilities will be available.

Applications should be sent to the Regius Professor of Physic, Department of Radiotherapeutics, Addenbrooke's Hospital, Cambridge, together with the names of two referees.

BRITISH LEATHER MANUFACTURERS' RESEARCH ASSOCIATION

Graduate in Chemistry or Biochemistry, or Dip.Tech. in Applied Chemistry required for research on composition of animal skins and chemical modifications of collagen. Work suitable for higher degree. Salary in range £950 by £75 to £1,850.

Applications to Director, BLMRA, Milton Park, Egham, Surrey.

UNIVERSITY OF OTAGO MEDICAL SCHOOL

DUNEDIN, NEW ZEALAND

LECTURER OR SENIOR LECTURER IN CHEMICAL PATHOLOGY

Applications are invited for the position of Lecturer or Senior Lecturer in Chemical Pathology. Salary according to experience and qualifications, within the following scales: Science graduate, £1,300 by £100 to £2,000 to £2,500; Medical graduate, £2,000 by £100 to £2,500 to £3,000. The status of Senior Lecturer may be awarded as a mark of personal distinction either on initial appointment or by subsequent promotion. The salary of a senior lecturer is not less than £2,300 for a science graduate and £2,700 for a medical graduate. Duties include teaching and participation in diagnostic services. The Department has an excellent technological staff. Research is encouraged and the appointee would have opportunity to study for a higher degree. Family passages and assistance with removal costs are provided. The appointee is expected to commence duties in March, 1966, or as soon thereafter as possible.

Further particulars are available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or from the Registrar of the University. Applications, with the names of three persons to whom reference can be made, close in New Zealand and London on December 15, 1965.

THE UNIVERSITY OF LEEDS. DEPARTment of Experimental Pathology and Cancer Research, School of Medicine, Leeds, 2. Two vacancies for technical assistants exist in the Electron Microscope Unit of the above department. The vacancies are for a Technical Assistant (precise grade according to qualifications and experience), and Junior Technician. The higher post involves electron microscope studies of a number of biological problems. The person appointed would be expected, after necessary training, to work largely alone. Ideally, the candidate should have H.N.C. or Dip.Tech., in applied Biology and some experience of electron microscopy. The junior post involves routine work and some experience in biology would be desirable. Salary in each case according to qualifications and experience.—Applications, giving particulars, to Dr. G. Eaves.

Glaxo Research Ltd. Fulmer, Bucks

Graduates in Biological Sciences

Vacancies exist for men and women with a good Honours Degree who are interested in work in the fields of steroid pharmacology or drug safety testing. Appropriate previous experience would be an advantage but is not essential.
The Company offers excellent conditions of employment including Pension and Bonus Schemes. It operates a 5-day 38-hour week. Amenities include a Cafeteria and Sports and Social Club.
Applications should be sent to the Personnel Officer (G.B.), Glaxo Laboratories Limited, Greenford, Middlesex.

THE WELLCOME FOUNDATION LIMITED

Chemical Development Laboratories

A chemist with a good honours degree and with an interest in synthetic organic chemistry and preferably, but not essentially, with some post-graduate experience is required in the Chemical Development Laboratories of the Wellcome Foundation Limited.

The Company is largely concerned with the discovery, manufacture and sale of new medical and veterinary products.

This position offers an opportunity for an enterprising young chemist to join an experienced team engaged in the development of processes for the manufacture of novel chemical substances from the laboratory through pilot plant to the full production scale.

Applications, quoting U.167, should be addressed to the Personnel Manager, the Wellcome Chemical Works, Dartford, Kent.

QUEEN MARY COLLEGE
(UNIVERSITY OF LONDON)
DEPARTMENT OF NUCLEAR ENGINEERING

Applications are invited for two posts of Lecturer. One should preferably be a Theoretical Reactor Physicist with experience in modern computational techniques and the other should be well qualified in the fields of fluid dynamics and heat transfer. Applications for the latter post need not necessarily have a nuclear engineering background. Departmental activities include research in the above fields as well as graduate and undergraduate teaching. Salary scale: £1,400 by £85 to £2,165 (efficiency bar) by £85 to £2,505 plus London allowance of £60 and F.S.S.U. participation. Initial salary according to qualifications and experience.

Application forms and further details obtainable from the Registrar, Queen Mary College, Mile End Road, London, E.1, to be returned by November 30, 1965.

CLARKSON COLLEGE OF TECHNOLOGY
POTSDAM, NEW YORK, U.S.A.

GRADUATE RESEARCH AND INSTRUCTIONAL ASSISTANTSHIPS for M.S. and Ph.D. candidates in CHEMICAL ENGINEERING available for February and September, 1966. Stipends $2,400 to $4,100 plus tuition for 12 months. Research in adsorption, crystallization heat transfer, reverse osmosis, mass transfer, reaction kinetics, boundary layer and other topics.

Applicants may contact: H. L. Shulman, Dean of the Graduate School, Clarkson College of Technology, Potsdam, New York, U.S.A.

UNIVERSITY OF CAPE TOWN
PROFESSOR IN MATHEMATICAL STATISTICS

Applications are invited for the position of Professor in Mathematical Statistics. The present salary scale is R.5,500 by R.200 to R.6,150. (Exchange rate: R.2=£1.) The initial salary will be dependent on the qualifications and experience of the successful applicant, who would be expected to commence his duties as soon as reasonably possible.

Two copies of the application, including details of academic training, experience in teaching and research, and giving the names of two referees, should reach the Secretary-General, Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, S.W.1 (from whom a detailed memorandum concerning teaching and research in Mathematical Statistics may be obtained) not later than November 15, 1965. A third copy of the application must be sent direct to the Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa, by the same date. The University reserves the right to appoint a person other than one of the applicants, or to make no appointment.

ANIMAL NUTRITIONIST
EAST AFRICAN AGRICULTURE AND FORESTRY
RESEARCH ORGANISATION

Required to investigate metabolism of tropical and temperate type cattle with a tropical environment.

Candidates should possess a degree in agriculture with postgraduate training or experience in biochemistry or agricultural biochemistry.

Appointment on contract for one or two tours of two years. Salary scale £1,221 to £2,808 a year. A 12½% increase in salaries has been approved. 25% terminal gratuity. Education allowances. Passages provided. Generous leave. Government quarters.

Applicants, who should be nationals of the United Kingdom or Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting PC213/214/016, to:

Appointments Officer,
Room 301,
MINISTRY OF OVERSEAS DEVELOPMENT,
Eland House,
Stag Place,
London, S.W.1.

UNIVERSITY OF WALES
UNIVERSITY COLLEGE OF SWANSEA

Applications are invited for the post of Research Assistant in the Department of Metallurgy to study the behaviour of point defects in metals using electron microscopy and electrical resistivity techniques. Suitably qualified applicants will be registered for the postgraduate degree. Salary according to age and experience. Further information from the Registrar, University College of Swansea, Singleton Park, Swansea.

VETERINARY OFFICERS
MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

PENSIONABLE posts in various parts of the country for men and women aged at least 23.

QUALIFICATIONS: Candidates must be M.R.C.V.S. with at least one year's practical post-graduate experience.

STARTING SALARY (national): £1,281 (at 23) to £1,719 (at 32 or over): scale maximum £1,902. Promotion to Grade I (£1,962 to £2,381 in 6 to 7 years for able officers, or possibly earlier, with prospects of further advancement. Opportunities may also arise for short-term service overseas.

WRITE to Civil Service Commission, Savile Row, London, W.1, for application form, quoting 130/65. Closing date November 19, 1965.

BIOCHEMISTS
BIOLOGISTS
PHARMACOLOGISTS

will be interested in the two opportunities we have to join our Technical Documentation Department which provides an efficient medical literature information service for our ten expanding marketing companies in Europe.

LITERATURE RESEARCH OFFICER

The Job:

(a) to assess trends in medical thinking and attitudes in specified areas of medicine and chemotherapy.

(b) to prepare surveys on selected topics.

(c) to screen, abstract, and evaluate world medical literature.

(d) to deal with technical enquiries involving information retrieval from departmental and external sources and to help maintain a number of indexes.

The Person:

21-25 years of age, male or female; must have a liking for desk research in medical/pharmaceutical subjects, and be able to present ideas in a forceful and compact way.

MANAGER– INFORMATION UNIT

The Job:

(a) to be responsible for the day-to-day operations of the Literature Research and Technical Enquiry and Indexing Sections of the department, controlling five graduates.

(b) to act as deputy to the Manager of the Technical Documentation Department and assist him in all matters.

The Person:

25-30 years of age; must be capable of supervising and training graduates, and experienced in information and editorial work; knowledge of indexing systems and information retrieval techniques is important.

The Company makes a wide range of quality preparations which are accepted and used throughout the medical profession. The range includes life saving drugs for hospital use, preparations for the treatment of serious illnesses, and products for the alleviation of minor complaints. We are constantly engaged upon research into new products designed to extend the conquest of disease and suffering.

Research results in expansion, which offers excellent future promotion prospects, both within the department and the company. Promotion and salary are determined by ability, not by age and experience.

Please write with details indicating your suitability for one of the posts advertised above—or for a Personal History Form, if this is more convenient–to the Staff Manager, The Winthrop Products Company, Winthrop House, Surbiton, Surrey.

Glaxo Research Limited
Stoke Poges, Bucks

Microbiologist

A vacancy exists in the Fermentation Research Department which should interest a good Honours graduate with an enquiring mind and the ability to pursue his own line of research. The work is concerned with a study of the effects of antibiotics with particular reference to their mode of action against bacteria. Some appropriate experience would be an advantage, but is not essential.
The Company offers excellent conditions of employment including Pension and Bonus Schemes. It operates a 5-day 38-hour week. Amenities include a Cafeteria and Sports and Social Club.
Applications should be be sent to the Personnel Officer (G.B.), Glaxo Laboratories Limited, Greenford, Middlesex.

TECHNICAL DIRECTOR

A British company, member of an International Group prominent in high vacuum technology and its application to a wide variety of processes, wishes to appoint a Technical Director. He will be responsible for leading teams concerned with generation, measurement, control and application of high vacua, and for developing and consolidating the Company's product range in collaboration with the commercial and production departments and with other Group technical interests.

We are seeking the following combination of attributes :

- A good qualification in engineering, physics or other appropriate discipline.
- Experience in the generation and/or use of high vacuum on an industrial scale.
- Evidence of maturity, leadership qualities and dynamism in a technological environment ; the preferred age range is 40 to 45.
- A strong commercial sense, and the ability to collaborate closely with production, sales and cost departments in securing the rapid and profitable introduction of new developments.

The Company regards this as a key appointment and is prepared to negotiate an attractive salary. It is unlikely that anyone currently earning less than £4,000 per annum would be suitably qualified.

Applications, which will be treated in strict confidence, should be made to :

URWICK, ORR & PARTNERS LTD.,
134 Buckingham Palace Road,
London, S.W.1,

who have been asked to advise on this appointment, quoting reference HS/2175 on the envelope.

TSETSE OFFICER
TANZANIA
(TANGANYIKA AND ZANZIBAR)

Duties include the administration of Tsetse fly survey and control projects. The officer will normally be based at one of the Regional Headquarters but will be required to spend long periods on safari in the "fly" country. Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should possess a degree in Biology, Zoology or entomology. Salary £1,329 to £2,757 per annum plus 25 per cent terminal gratuity. Education allowances. Free pasages. Quarters available. One or two tours of 21 to 27 months.

Please apply for further details, giving full name, qualifications, experience and quoting Ref.: RC 217/173/05 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

CITY OF BELFAST COLLEGE OF TECHNOLOGY

Principal: D. H. Alexander, O.B.E., M.Sc.(Cantab.), Wh.Sch., M.I.Mech.E.

Applications are invited for the following position:

LECTURER IN FOOD SCIENCE

Salary: £1,670 to £1,895 (under review). Applicants should hold an honours degree or an equivalent qualification, in food science, microbiology, chemistry applied to food, or other similar subjects. Industrial or research experience is desirable and would be an added qualification.

Further particulars and form of application may be obtained from the Principal, College of Technology, Belfast 1, with whom applications must be lodged not later than Thursday, November 4, 1965.

W. C. H. EAKIN,
Director of Education.

AUSTRALIAN NATIONAL UNIVERSITY
INSTITUTE OF ADVANCED STUDIES
DATA CONTROL ENGINEER

Applications are invited for appointment as Data Control Engineer in the Department of Nuclear Physics in the Institute of Advanced Studies.

Qualifications: A sound knowledge of digital electronics, logic and computer programming is required.

Duties: The successful applicant will be required to assist in the development of electronic equipment enabling the transfer of data and control signals between nuclear physics apparatus and an IBM 1800 Data Acquisition and Control Unit. Responsibility will be taken for the modification of existing equipment, where suitable, and for the design and construction of new equipment. In addition, the appointee will be expected to assist in the maintenance of this equipment and in the development of suitable control programmes for the system.

Salary and conditions of appointment: The salary will be determined within the range £A2,400 to £A3,150. The appointment will be for three years with an extension to five years possible in certain circumstances. Superannuation is on the F.S.S.U. pattern with supplementary benefits. Reasonable travel expenses are paid and assistance with housing is provided.

Further particulars should be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 26, 1965.

UNIVERSITY OF LONDON
READERSHIP IN STATISTICS AT THE LONDON SCHOOL OF ECONOMICS

The Senate invite applications for the Readership in Statistics tenable at the London School of Economics and Political Science (salary scale £2,450 to £3,250 a year plus £80 London Allowance).

Applications (ten copies), must be received not later than November 18, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

KING'S COLLEGE HOSPITAL
DENMARK HILL, S.E.5

Applications are invited for a Junior Physicist. The successful applicant will undertake duties as radiological protection adviser in several hospital groups in the South-east Metropolitan Regional Hospital Board, but will spend most of his time at K.C.H., where he will join in the general work of the department in radioisotopes in radiotherapy, physics, and in medical electrons. Some opportunities will be available for research. Some experience in protection work would be desirable, but training can be given. Salary between £875 and £1,665 depending on qualifications and experience.

Further details can be obtained from the Director of the Department. Applications, including the names of two referees, should be sent before November 13 to the House Governor.

HOUGHTON POULTRY RESEARCH STATION
(Financed jointly by the Agricultural Research Council and the Animal Health Trust)

HEAD OF PHYSIOLOGY AND BIOCHEMISTRY DEPARTMENT

A senior research worker, preferably holding veterinary or medical qualifications, is required to fill the new post of head of the Station's combined physiology and biochemistry department. The work of this expanding department will be linked to the main theme of the Station's programme, i.e. avian diseases. The successful applicant will have the opportunity of designing the future programme of the department.

The appointment will be made within the Senior Principal Scientific Officer scale (£3,300 per annum to £3,800 per annum). Superannuation under F.S.S.U.

Further information from the Station Secretary, Houghton, Huntingdon, to whom applications should be submitted by November 30. It is hoped to conduct interviews in London in mid-December.

ELECTRICAL PROPERTIES OF SOLIDS

MORGANITE RESEARCH AND DEVELOPMENT LIMITED,
Battersea Church Road,
London, S.W.11

Morgan

PHYSICIST required to study the electrical properties of materials, in particular glazed ceramics and multiphase systems.

EXPERIENCE of techniques for studying the structure of materials in relation to their electrical properties would be an advantage.

QUALIFICATIONS would include a good honours degree and post-graduate experience in a relevant field.

CONDITIONS of employment will attract the right man. The work is based on the new Northfields laboratories, overlooking Wandsworth Park, S.W.18.

APPLY to the Staff Manager at Battersea Church Road, quoting reference N/QG10.

OXFORD UNIVERSITY

PHYSICIST or ELECTRONICS DEVELOPMENT ENGINEER

required to join an existing bubble chamber film analysis group, to develop apparatus for measuring tracks on bubble chamber photographs, both by computer-controlled manual techniques and by a fully automatic computer-controlled system. A degree, or previous experience in the field, is required. Salary in the range £1,600 to £2,300; starting point dependent on age and experience.

RESEARCH ASSISTANTS

required by the same group, to supervise scanning and measuring staff, to organize the recording of data derived from bubble chamber photographs, and to prepare programmes for computer processing of the data. Salary in the range £1,000 to £1,750 (with a higher ceiling for graduates); starting point dependent on age and experience.

Applications to Professor D. H. Wilkinson,
NUCLEAR PHYSICS LABORATORY,
21 Banbury Road, Oxford.

UNIVERSITY OF GLASGOW
COMPUTING DEPARTMENT

Applications are invited for the post of Lecturer (Computer Manager) at a salary of £2,165 to £2,505 per annum, from graduates with experience of computer systems and administration. The person appointed will be responsible to the Director for the operation of the University's KDF 9 computer.

Applications (eight copies), should be lodged, not later than November 15, 1965, with the undersigned, from whm further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

PLYMOUTH GENERAL HOSPITAL. Radiotherapy Department. Physics Technician (basic or senior grade) required to assist in some of the routine work with electronic and isotope equipment, and possibly additional technical duties in radiotherapy. Qualifications required: O.N.C. in electrical engineering or applied physics or equivalent. Salary scale £668 to £1,061 (at present under review) according to qualifications and experience.—Further details may be obtained from the Principal Physicist, Radiotherapy Department, Plymouth General Hospital, Plymouth.

UNIVERSITY OF ILLINOIS
COLLEGE OF PHARMACY
833 SO. WOOD STREET, CHICAGO, ILLINOIS 60612

Applications are invited for the post of Instructor or Laboratory Assistant in Pharmacognosy for the Academic Year 1966-67. Applicants should have M.S. or be approaching Ph.D. Salary commensurate with training and experience. Give complete Educational History and experience.

Applications should reach Dr. Ralph F. Voigt, Head of Department of Pharmacognosy and Pharmacology by November 15, 1965.

SUTHERLAND DENTAL SCHOOL
NEWCASTLE UPON TYNE

Graduate in Organic Chemistry or Biochemistry required to take part in research on dental decay and its prevention by fluoride. Salary up to £850.

Applications, with details of training and the names of two referees, to Professor G. N. Jenkins, Department of Oral Physiology, Sutherland Dental School, Newcastle upon Tyne, 1.

UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF PURE MATHEMATICS

Applications are invited for the Chair of Pure Mathematics which has become vacant through the resignation of Professor Bonsall on his appointment to the M'Laurin Chair at Edinburgh University. The person appointed will be required to take up duty as soon as possible and not later than August 1, 1966. Salary in accordance with professorial scale (£3,400 by £125 to £4,025 per annum) with membership of F.S.S.U.

Further particulars may be obtained from the undersigned with whom applications (15 copies), giving the names of not more than three persons to whom reference may be made, must be lodged not later than Saturday, November 20, 1965. (Applicants from outside the British Isles may submit one copy only.)

E. M. BETTENSON,
Registrar.

University of Newcastle upon Tyne,
6 Kensington Terrace,
Newcastle upon Tyne 2.

UNIVERSITY OF MANITOBA
invites inquiries from persons interested in

(1) Staff positions as nuclear theorists, with at least partial interests in nuclear reactions.
(2) Staff positions as solid state theorists, with interests in some aspects of magnetism.
(3) Post-doctoral Fellowships—to work in proton-induced reactions (50 MeV); alpha, beta and gamma ray spectroscopy; hyperfine structure; cosmic rays; magnetism; positronium; mass spectroscopy.

Inquiries should be addressed to Professor H. E. Duckworth, Acting-Head of the Department of Physics, Winnipeg 19, Manitoba, Canada.

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY
(Faculty of Technology in the University of Manchester)

An Experimental Officer is required to take charge of the polymer Technology Laboratory and conduct research in this field under the direction of a member of staff. The post would be suitable for a person with at least two years responsible practical experience in the fields of either polymer technology, physics, physical chemistry or light engineering and candidates are expected to be of H.N.C. standard. Commencing salary according to qualifications and experience in the scale £870 by £50 to £1,270 by £60 to £1,450 per annum. Superannuation Scheme.

Application forms and further details may be obtained from the Registrar of the College, Sackville Street, Manchester, 1, to whom completed applications should be returned by November 13, 1965.

AUSTRALIA

CHEMISTS, PHYSICISTS or ANALYTICAL CHEMISTS

B.H.P. CENTRAL RESEARCH LABORATORIES, SHORTLAND, N.S.W.

THREE POSITIONS

Duties: To take responsibility for sub-groups within the Analytical Chemistry Section. The section is presently organized into a number of sub-groups and expansion has created several vacancies at the Research Officer level for men or women with initiative. Appointees will receive some freedom to develop topics of their own choice and publication is encouraged. The Analytical Chemistry Section is well equipped and specialist supporting services are available within other sections.

(i) Direct Reading Vacuum Emission Spectrometry. This sub-group services the varied research projects dealing with appraisal of new ore-bodies, mineral dressing, pyrometallurgy and properties of ferrous metals. The successful appointee will be required to initiate geochemical and trace element studies.

(ii) Fuel Analyses. This sub-group carries out comprehensive analytical studies of coal exploration and fundamental fuel investigation samples.

(iii) This sub-group is responsible for the study, identification and analyses of inclusions in ferrous metals, gases in metals, and phases in raw materials.

Applications from recent graduates who may wish to work within the various sub-groups will be considered. The Company's expansion programme affords excellent opportunities for progress and advancement.

Qualifications: No particular academic course is specified but possession of a professional qualification in a scientific field is essential. Higher academic qualifications and experience would be an advantage.

Salary: This will be negotiated on the basis of qualifications and experience, but for the positions offered it is envisaged that appointments will be made in the range £A2,000 to £A2,500 per annum. However, initial appointments may be made outside this range.

Passage to Australia: Passages for the successful applicants and their dependent families will be arranged under the assisted passage scheme where applicable. For married men, financial assistance will be given towards resettlement in Australia.

General: Admission to superannuation fund follows a short probationary period when officers are also covered by free temporary life assurance. Sickness benefits are liberal. Annual leave is four weeks and, in addition, public holidays are observed. The positions are at the Company's Central Research Laboratories at Shortland, a suburb of Newcastle on the N.S.W. Central Coast, 100 miles north of Sydney. Educational facilities in the area are excellent and include a University and a Technical College.

Applications, stating age, qualifications, experience and references, should be marked "Confidential" and addressed, quoting U25, to: Research Manager, The Broken Hill Proprietary Co. Ltd., Central Research Laboratories, Shortland, 2N, N.S.W., Australia.

RESEARCH *Vacancies exist for:*

PHYSICAL METALLURGISTS

—B. H. P. AUSTRALIA

Duties: To participate in a study of properties of steel ranging from fundamental to developmental work associated with new steels.

Qualifications: University degree in Applied Science (preferably metallurgy) is a necessity, while a higher degree (M.Sc. or Ph.D.) or equivalent research experience is desirable and experience in one or more of the following fields would be an advantage—creep and elevated temperature tensile testing; oxidation; properties of carbon and low alloy steels; steel evaluation; brittle fracture.

Salary: To be negotiated on the basis of qualifications and experience within the range £A2,000 to £A2,500 per annum. Appointments may however be made outside this range.

Location: The laboratories are located in a suburb of Newcastle, a city with an urban population of approximately 250,000 on the central coast of New South Wales, 100 miles north of Sydney. Excellent recreational facilities abound within the Newcastle district.

Company: B.H.P. operates iron and steel-making facilities with an installed capacity in excess of 5,000,000 ingot tons per annum. This progressive Company and its subsidiaries are undergoing rapid expansion in fields covering many products, from wire and tinplate manufacture to shipbuilding, and including special steels and hard metals.

General: Appointment is subject to satisfactory medical fitness. Appointees will be entitled to membership of a contributory superannuation scheme, four weeks annual leave, personal life and hospital and medical cover, plus other normal staff conditions. Transport for appointees and their dependent families will be arranged. For married men assistance can be given towards a suitable housing loan.

Applications, stating age, qualifications, experience and referees, should be addressed (quoting U.24) to: Research Manager, The Broken Hill Proprietary Co. Ltd., Central Research Laboratories, Shortland, N.S.W., Australia.

UNIVERSITY OF CAPE TOWN

LECTURER IN MATHEMATICAL STATISTICS

Applications are invited for the position of Lecturer in Mathematical Statistics. The present salary scale is R.3,200 by R.150 to R.4,100 per annum. (Exchange rate: R.2=£1.) The initial salary will be dependent on the qualifications and experience of the successful applicant who would be expected to commence his duties as soon as reasonably possible.

Two copies of the application, including details of academic training, experience in teaching and research, and giving the names of two referees, should reach the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (from whom a detailed memorandum concerning teaching and research in Mathematical Statistics may be obtained), not later than November 15, 1965. A third copy of the application must be sent direct to the Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa, by the same date. The University reserves the right to appoint a person other than one of the applicants, or to make no appointment.

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

SCHOOL OF ENGINEERING

Applications are invited for the post of LECTURER IN ELECTRICAL ENGINEERING from candidates with good academic qualifications and postgraduate experience. Applicants should have a special interest in the analysis and design of power generation and distribution systems, and a familiarity with modern methods of analysis and simulation of systems, using computer techniques, would be appropriate.

Salary will be on the scale £1,400 by £85 to £2,505 per annum, together with F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, to whom applications should be received by Friday, November 12, 1965.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

NATIONAL AGRICULTURAL ADVISORY SERVICE

GRADE B OFFICER (UNESTABLISHED)
required for
ANALYST POST
at Bangor, North Wales

Male or female, at least 20 years of age on December 31, 1965. Duties include examination and analysis of feeding stuffs, plant materials, soils and other agricultural materials. Candidates should normally possess G.C.E. "O" level in English language and four other subjects, including "A" level in two science subjects, one of which must be chemistry, or H.N.C. in chemistry, or L.R.I.C., or pass degree in chemistry or equivalent. Experience in analysis of agricultural materials an advantage. Starting salary £644 (at 20) to £962 (at 26 or over)—scale maximum £1,195. Opportunities for promotion.

Applications to Establishment Officer, Ministry of Agriculture, Fisheries and Food, Welsh Department, Plas Crug, Aberystwyth, by November 30, 1965.

HERTFORDSHIRE COUNTY COUNCIL

HERTIS

Applications are invited from qualified librarians or information officers with wide industrial, research, or college library experience for the post of Assistant County Technical Librarian, based at Hatfield College of Technology. HERTIS (Hertfordshire Technical Library and Information Service) comprises the libraries of colleges of technology and further education in the county, together with an information service for industry. The post offers scope for initiative in developing a modern and rapidly expanding service. Salary within the Burnham scale for Senior Lecturers: £2,140 to £2,380.

Further details from the Registrar, Hatfield College of Technology, Hatfield, Herts.

SK&F

LABORATORY ASSISTANT

in Biological Group

Smith Kline & French Laboratories Limited are engaged in an extensive biological research programme concerned with the discovery and development of pharmacologically active substances.

We are looking for a laboratory assistant to work within the biological research group who has had experience in the use of microbiological and/or tissue culture techniques and who wishes to specialize in the latter field. He or she should be 25 years of age and over, preferably with A.I.M.L.T. or F.I.M.L.T. and who is capable of working on his or her own initiative without close supervision.

Working conditions are first class and include:
- Good starting salary, reviewed annually on merit.
- 5-day week. 9-4.45 p.m.
- Excellent cafeteria.

Please write for an application form to:
The Assistant Personnel Officer,
SMITH KLINE & FRENCH LABORATORIES LIMITED,
Mundells, Welwyn Garden City, Herts.

quoting Reference No. 06/70/43.

California & Texas

GEOPHYSICAL RESEARCH STAFF

Salaries from $12,000 to $20,000

As a consequence of continuous expansion, one of the largest American companies engaged in world-wide Geophysical Exploration for the major oil companies has several attractive vacancies on its Research Staff:—

HEAD OF GEOPHYSICAL RESEARCH in California, to direct the work of the Research Group and of a number of consultants. He will concentrate on long range programmes involving specific ray and wave analyses, data processing techniques utilizing digital computers, information theoretic approaches to seismic data analysis and development of novel geophysical sources and receivers. He must have a proven record of accomplishment. Salary about $20,000.

SENIOR GEOPHYSICIST at Houston, Texas, to provide leadership and guidance for the R & D Group and to concentrate on short term programmes. He should have the experience and ability to succeed the Head of the Group in a few years. Salary about $16,000.

GEOPHYSICISTS for the Research Staffs at Houston and in California. Salary not less than $12,000.

Age is not critical but applicants must have a Ph.D. or good honours degree in Physics, some experience in exploratory geophysics and familiarity with modern digital processing techniques.

There are very good fringe benefits and relocation expenses will be paid.

Please write in confidence, to:—
J. P. Hope (Ref: H/676/162),
Overseas Appointments,
P-E Consulting Group
12, Grosvenor Place, London, S.W.1.

LANCHESTER COLLEGE OF TECHNOLOGY
COVENTRY

Glass-blower required for the Department of Applied Physics. Candidates should have had appropriate training, together with some experience of high vacuum work and chemical glass blowing. The successful applicant will be appointed technician in the Department of Applied Physics. Salary: Within the range £630 to £1,050 per annum. Commencing salary depends on age and experience. Additional £26 payable in certain circumstances. Permanent post in the service of Coventry Corporation, with superannuation, staff status, monthly salary, generous sick leave and holiday schemes. Canteen available.

Application forms from Principal, Lanchester College, Priory Street, Coventry, returnable by November 8, 1965.

UNIVERSITY OF MALAYA
CHAIR OF ZOOLOGY

Applications are invited for appointment to the above-mentioned Chair. Candidates should have in addition to high academic qualifications, experience in teaching at University level and, preferably, experience in the administration of a department.

Emoluments: The amounts mentioned below refer to Malayan dollars (per mensem) and their approximate sterling equivalents (per annum) are shown in brackets. Salary: There is a range of basic salaries, to one of which a Professor is appointed, depending on his qualifications and experience. These are: $1,770 (£2,478), $1,830 (£2,562), $1,890 (£2,646), $1,950 (£2,730), $2,010 (£2,814) $2,070 (£2,898), $2,130 (£2,982). In addition, the following allowances are payable: Variable allowance: 35 per cent of basic salary, subject to certain maxima depending on marital status: minimum $150 per mensem (£210 per annum), maximum $400 per mensem (£560 per annum). Inducement allowance: (If awarded), up to $1,000 (£1,400) per mensem. (In exceptional cases an inducement allowance exceeding $1,000 per mensem may be granted).

Housing will be provided, if available, for which a rent of $100 to $125 per mensem (£117 to £175 per annum) will be charged.

Provident Fund: Staff member contributes 5 per cent and university 15 per cent of basic salary.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. The closing date for the receipt of applications in London and Kuala Lumpur is November 30, 1965.

HEWLETT hp PACKARD / Appointments

F & M SCIENTIFIC DIVISION

The new chemical instrumentation division of Hewlett-Packard Ltd. is expanding rapidly. A marketing organisation for gas chromatographs, osmometers and other chemical instrumentation is being built up.

Immediately, we need:

An **APPLICATIONS CHEMIST** with B.Sc. (Hon.) and several years' G.L.C. analytical experience in the organic or biochemical fields. Ability to develop G.L.C. methods, to demonstrate instruments to groups of customers and to instruct customers in their use is essential. Age 28–40. All experience and any publications will be taken into consideration. Based in London.

A TECHNICAL SALES REPRESENTATIVE with B.Sc. or equivalent, G.L.C. experience and preferably other analytical experience. Age 25–35

An **INSTRUMENT SERVICE ENGINEER** to install and service instruments, especially gas chromatographs, in customers' laboratories. Basic understanding of electronics with qualification to H.N.C. (Elec) is desirable, but several years' experience of this type of instrument work might be acceptable. Age 24–35.

Company benefits include a non-contributory pension and life assurance scheme, profit sharing and Company stock purchase scheme. In the cases of (2) and (3) above, a Company car would be provided.

Interviews will be in Bedford initially and later in London. Training will be in London, Amsterdam and probably Geneva.

Write, giving complete personal details, qualifications and experience to:

THE MARKETING MANAGER
HEWLETT-PACKARD LTD.,
DALLAS ROAD, BEDFORD.

KENT EDUCATION COMMITTEE

MEDWAY COLLEGE OF TECHNOLOGY

DEPARTMENT OF SCIENCE

Vacancies have arisen for 1 SENIOR LECTURER and 1 LECTURER in the Biology Section of the Department. Applicants for these posts should have specialist qualifications and postgraduate experience in one of the following fields: (i) Biochemistry. (ii) Animal or Plant Physiology. (iii) Microbiology.

LECTURER IN PHYSICAL CHEMISTRY required to teach to B.Sc. and Grad.R.I.C. Part II level. Applicants should be well qualified academically and have postgraduate experience in industry and/or teaching.

The successful candidates will be given the opportunity to undertake research, for which good facilities are available.

Salaries (subject to ratification): Senior Lecturer, £2,140 to £2,380; Lecturer, £1,875 to £2,140.

Application forms and further details from the Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

TWO TEACHING FELLOWS/ DEMONSTRATORS IN AGRONOMY

Applications are invited for the above positions. Applicants should be graduates, preferably with an Honours Degree in Rural Science, Agriculture or Science with specialization in Plant Sciences. Additional training in Soil and Animal Science would be an advantage. The successful applicant may be required to give a short course of lectures as well as to demonstrate in one of soil, crop or pasture aspects of Agronomy. A teaching fellow will be required to enrol for a higher degree. Commencing salary will be determined according to qualifications and experience within the range of £A1,500 by £A100 to £A1,900 per annum. Fares will be paid to the successful applicants and a contribution will be made towards removal expenses.

Further information may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 26, 1965.

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

LABORATORY TECHNICIAN OR SENIOR LABORATORY TECHNICIAN

Applications are invited for the above-mentioned position, the grade of appointment being dependent upon experience and qualifications. The successful applicant is required for research and routine class work in the Sections of Parasitology, Pathology and Preventive Medicine in the School of Rural Science. Duties will centre on immunological and parasitological techniques and may include other experimental work. Applicants should possess technical qualifications, I.M.L.T. or I.S.T./C.G.L.I., and previous biological experience is essential. Commencing salary for the position of Laboratory Technician will be within the range £A1,190 to £A1,440 per annum, and for a Senior Laboratory Technician within the range £A1,594 to £A1,705 per annum. Conditions of employment include four weeks' recreation leave per annum, excellent sick and long service leave benefits. Participation in the Superannuation and Housing Scheme is possible after a qualifying period. The successful applicant would be expected to travel to Australia under the assisted passage scheme.

Applications should include personal information, full details of qualifications and experience and also the names of two people to whom reference may be made on the applicant's behalf, and should reach the undersigned no later than November 30, 1965.

T. C. LAMBLE,
Registrar.

Armidale,
N.S.W., Australia.

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

LECTURER IN PSYCHOLOGY

Applications are invited for the position of Lecturer in Psychology. Interest and competence in physiological and animal psychology would be an advantage. Commencing salary will be determined according to qualifications and experience within the range of £A2,400 by £A110 to £A3,170. Provision is made for superannuation, travel and removal expenses and for assistance in buying or building a home.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 22, 1965.

UNIVERSITY OF GLASGOW

ASSISTANTSHIP IN VETERINARY CLINICAL BIOCHEMISTRY

Applications are invited for an Assistantship in Veterinary Clinical Biochemistry. Salary scales: £1,050 to £1,275 per annum. Initial salary according to experience and qualifications. F.S.S.U.

Applications (three copies), should be lodged, not later than November 10, 1965, with the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

The Department of Science and Technology, the Architectural Association, London, is inviting applications for the post of

WORKSHOP MANAGER

in the Faculty of Building Science, Architure and Planning, University of Science and Technology, Kumasi, Ghana.

Applicants should be capable of organizing and training workshop and laboratory staff, instructing students in model making, maintaining scientific equipment, workshop machinery, and stores.

—contract appointment for five years or less

—passage paid, home leave for manager and his family

—low rent house, generous allowances

—salary in the region of £1,500 per annum according to qualifications and experience.

Applications, stating age, qualifications, experience, and names and addresses of two referees, to the Senior Registrar, Architectural Association School of Architecture, 36 Bedford Square, London, W.C.1.

MONASH UNIVERSITY
MELBOURNE, AUSTRALIA
SENIOR TEACHING FELLOW IN INORGANIC CHEMISTRY

Applications are invited for the above position in the Department of Chemistry. The appointee will be required to conduct tutorials and practical classes for undergraduates (mainly at an advanced level) and may participate in research currently under way in one of the following fields: organometallic chemistry (perfluoro and perchlorocarbon derivatives of the elements), silyl chemistry, transition metal chemistry (electron transfer reactions, stereo-chemistry, magneto chemistry, X-ray structural studies). Preference will be given to applicants who hold, or are completing, a Ph.D. degree and who have had teaching or research experience in inorganic chemistry. Candidates having other qualifications may be considered for a Teaching Fellowship. Salary ranges: Senior Teaching Fellow, £A1,900 to £A2,150 per annum; Teaching Fellow, £A1,600 to £A1,750 per annum, with superannuation provision on the F.S.S.U. basis for Senior Teaching Fellows only. The appointment is for one year initially but is renewable for two further years.

Further general information and details of application procedure are available from the Registrar of the University, P.O. Box 92, Clayton, Victoria, Australia or the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Technical inquiries should be addressed to the Professor of Inorganic Chemistry, Professor B. O. West, in the University. Closing date: November 30, 1965.

THE UNIVERSITY OF SUSSEX
TECHNICIAN

A research TECHNICIAN is required shortly to assist Professor J. F. Sutcliffe in an investigation of the absorption and movement of mineral salts in intact plants. Applicants should preferably have some experience in growing plants under controlled conditions and of chemical analysis.

Salary according to qualifications and experience up to a maximum of £950 per annum. The period of appointment will be two years in the first instance with the possibility of extension.

Applications (three copies), giving details of age, qualifications and experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Establishment), the University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, as soon as possible.

GEOLOGISTS AND GEOPHYSICISTS

Hunting Geology and Geophysics Ltd. is a vigorous and growing company offering a variety of services to its clients and an interesting and rewarding career to its staff.

Required immediately are two GEOPHYSICISTS who are keen on variety and who have interest or experience in one or more of the following: seismic, resistivity or total force magnetics.

Required in two to three months is a third geophysicist with similar qualifications and a GEOLOGIST with ground water experience.

For all posts the preferred age is 22 to 32 and a good honours degree is essential.

Applications should be sent to:

Personnel Manager,
HUNTING SURVEYS & CONSULTANTS LTD.,
6 Elstree Way,
Boreham Wood, Herts.

CHIEF TECHNICIAN TO TAKE CHARGE of chemical laboratories and stores in Chemical Engineering Department. Some knowledge of glassblowing and experience with instrumental analysis, e.g., gas chromatography, an advantage but not essential.—Application forms from Establishment Officer, University College London, Gower Street, W.C.1, quoting Chem.Eng/5.

R. & W. PAUL (MALTSTERS) LTD.
require a

GRADUATE BIO-CHEMIST

This is a new appointment and the successful candidate will be deputy to the Chief Development Chemist, but will have particular responsibility for the development of novel enzymic conversions connected with sweeteners and malt products. He will have full opportunity, under general guidance, of developing his own methods.
Applicants should be in their 20's. The initial salary will be established in the range £1,200 to £1,700, according to postgraduate experience. There are opportunities for promotion within the group of companies, and conditions of service are excellent.
Applications, giving brief personal details, should be addressed to Mr. C. S. Drake, Pauls & Whites Ltd., P.O. Box 39, Key Street, Ipswich, quoting Reference BC1.

WYETH

Microbiologist

An opportunity has arisen for a microbiologist to join an active microbiological research unit within the Research and Development Division.

The successful candidate will be a qualified bacteriologist or microbiologist who is interested in the evaluation of potential chemotherapeutic agents or of pharmaceutical formulations.

This appointment is located at the Company's modern laboratories situated in pleasant surroundings at Havant, Hants, and offers a good commencing salary and future prospects. A non-contributory pension scheme is in operation.

Please write in the first instance to:

Personnel Manager, John Wyeth and Brother Limited,
Huntercombe Lane South, Taplow, Maidenhead, Berks.

UNIVERSITY OF ALBERTA
DEPARTMENT OF CHEMISTRY

Applications are invited for the position of

PROFESSOR in Physical Chemistry

Candidates in the fields of Solid State, Structure of Liquids, Spectroscopy or Thermodynamics are favoured, but all applications will be considered. Starting salary according to qualifications, in the range $15,000 to in excess of $20,000. Facilities for research are excellent and generous support is obtained from Provincial and National sources. The Department has 30 members and is active in all the major divisions of chemistry.

Further particulars may be obtained from

Professor Harry E. Gunning, Head,
Department of Chemistry,
University of Alberta,
Edmonton, Alta., Canada.

FISONS PHARMACEUTICALS LIMITED

GRADUATE BACTERIOLOGIST

FISONS PHARMACEUTICALS LIMITED wish to appoint a graduate in bacteriology (or equivalent), preferably with a few years' experience, to the staff of the Microbiology Department of its Research Laboratories. The work involves various and interesting aspects of pharmaceutical microbiology and the assessment of new chemotherapeutic products. The successful candidate will also be responsible to the head of the department for the training and supervision of junior technical staff.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

FISONS PHARMACEUTICALS LIMITED

MICROBIOLOGIST

FISONS PHARMACEUTICALS LIMITED wish to appoint a Microbiologist, preferably with some experience, to the staff of the Microbiology Department of its Research Laboratories. The work involves various and interesting aspects of pharmaceutical microbiology, including production control and the assessment of new chemotherapeutic compounds.

Candidates (male or female) should possess at least the Intermediate I.M.L.T. or be taking the H.N.C. course in Applied Biology with preference for bacteriology.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY

Applications are invited for the above position. The Department of Biochemistry and Nutrition is responsible for teaching biochemistry in the Faculties of Science and Rural Science. Applicants should state whether they seek a permanent or short-term appointment. Commencing salary will be determined according to qualifications and experience within one of the ranges: Senior Lecturer, £A3,300 by £A100 to £A3,800; Lecturer, £A2,400 by £A110 to £A3,170. In the case of a permanent appointment, provision is made for superannuation, travel and removal expenses, assistance in buying or building a home, and for study leave. Study leave travel grants are available and credit may be given for existing study leave entitlement. Short-term appointees are allowed travel and removal expenses to Armidale and, for return travel and removal expenses, the University will contribute up to a maximum of £A400.

Further information may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on November 26, 1965.

UNIVERSITY OF LEEDS

Applications are invited from graduates in physics or electrical engineering to assist in the application of video-type recording techniques for a post of Research Assistant and photographing recording to clinical diagnostic radiology. The work will be carried out in the Departments of Radiodiagnosis and Medical Physics, where research into many aspects of image structure and processing is already proceeding. The appointment is for one year and salary will be up to £1,200, depending upon qualifications and experience.

Applications (three copies), stating age, qualifications and experience should reach the Registrar, the University, Leeds, 2, not later than November 15, 1965.

Electron Microscopy and Diffraction Techniques

Applications are invited from Senior Scientists experienced in the application of Electron Microscopy and Electron Diffraction Techniques to the study of the structure of solids.

Electron microscopy is being increasingly used to further our research on the role of both lattice defects in crystals and micro structure in general, as well as on the physics and chemical properties of materials in bulk and thin film form.

Experience of dislocation physics, the strength of solids and diffraction theory would be an advantage.

This is a senior appointment within the Research Department of the company. A realistic salary will be paid and applications from scientists living abroad are welcome.

Apply, in confidence,
to the Staff Manager,
MORGANITE RESEARCH AND DEVELOPMENT LIMITED,
Battersea Church Road,
London, S.W.11.

UNIVERSITY OF LEEDS

Applications are invited from graduates in physics or electrical engineering for a post of Research Assistant to carry out development work in the field of image intensifiers and television applied to clinical diagnostic radiology. The work will be carried out in the Department of Medical Physics, where research into many aspects of image structure and processing is already proceeding. Candidates should preferably have postgraduate experience in a branch of electronics, e.g., in image tube measurements, television technology, etc. The appointment will be for an initial period of three years. Initial salary within the range £1,200 to £1,500.

Applications (three copies), stating age, qualifications and experience, should reach the Registrar, the University, Leeds, 2, not later than November 15, 1965.

DUBLIN INSTITUTE FOR ADVANCED STUDIES

SCHOOL OF COSMIC PHYSICS

EXPERIMENTAL OFFICER AT DUNSINK OBSERVATORY, DUBLIN

Applications are invited for the above post of Experimental Officer from persons with several years' experience in the design and construction of electronic equipment or with extended experience in the maintenance of optical, elctronic or data-processing equipment. Formal qualifications will be expected, but candidates without a university degree will be considered. The salary range is £1,400 by £60 to £1,740. A non-contributory pension scheme and family allowances are attached to this post.

Applications should reach the Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin, 4, on or before November 20, 1965.

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

DEPARTMENT OF PHYSICS

Applications are invited from graduates in Physics for the post of SUPERINTENDENT OF LABORATORIES in the Department of Physics. The appointment carries responsibility for administration of expanding new facilities, and preference will be given to candidates with experience of administration in industry, in the government service or in universities.

Salary will be on the scale £1,400 by £85 to £2,505, together with F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, by whom applications should be received by Friday, November 12, 1965.

THE ROYAL VETERINARY COLLEGE

(University of London)

The College proposes to make an appointment to a Lectureship in the Department of Pathology. Preference will be given to an applicant with a veterinary qualification who is interested in chemical pathology. Salary scale £1,400 by £85 to £2,165 then, subject to review, by £85 to £2,505 per annum plus £60 per annum London allowance. Commencing salary within the scale according to experience and qualifications. Superannuation benefits under F.S.S.U.

Application forms may be obtained from the Secretary, The Royal Veterinary College, Royal College Street, London, N.W.1, and should be returned not later than November 30, 1965.

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PURE MATHEMATICS

Applications are invited for the post of Lecturer or Assistant Lecturer in Pure Mathematics to begin duties on October 1, 1966. Preference will be given to candidates who have specialized in Modern Analysis or Topology, but applications from mathematicians whose interests lie in other branches of Pure Mathematics will also be considered. Initial salary according to qualifications and experience on the scales: Lecturer, £1,400 by £85 to £2,505; Assistant Lecturer, £1,050 by £75 to £1,275 with F.S.S.U. provision.

Further particulars may be obtained from the Registrar, to whom applications (four copies) should be sent by November 20, 1965.

NEW ZEALAND

AGRICULTURE DEPARTMENT

Applications are invited for the undermentioned vacancy:

Vacancy B 13/2/54/4111

SCIENTIST

Levin

Salary: Up to £2,710 per annum according to qualifications and experience.

Qualifications desired: Bachelor of Science (Agricultural, horticultural or plant science) with major units in Plant Physiology, Botany or Agricultural Botany, Physics; knowledge of crop protection, field experimental work with horticultural crops, biometrical method and agricultural engineering; particularly sprinkler irrigation systems, an advantage.

Duties: Research on irrigation and water relations of horticultural crops, especially outdoor vegetables. Methods and timing of water applications in relation to water-sensitive periods of various crops. Estimation and measurements of water requirements and relationship to climatological data and soil types, including special study of soil moisture measurements and distribution in relation to root systems of horticultural crops. Officer will require to have knowledge of performance and installation of irrigation equipment and be able to test systems. He will advise Advisory Officers and prepare material for departmental and public presentation. Some travel to districts to study irrigation problems.

Passages: Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses: Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, with whom applications will close on November 19, 1965.

Please quote reference B 13/2/54/4111 when inquiring.

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF CHEMISTRY

Applications are invited for two or more LECTURESHIPS or ASSISTANT LECTURESHIPS in Chemistry for October, 1966.

Further particulars may be obtained from the Secretary, University of Lancaster, Bailrigg House, Lancaster, to whom applications should be sent so as to arrive by January 1, 1966.

UNIVERSITY OF LONDON

READERSHIP IN NUCLEAR ENGINEERING AT QUEEN MARY COLLEGE

The Senate invite applications for a Readership in Nuclear Engineering (with special interest in either experimental reactor physics, fluid dynamics or heat transfer) tenable at Queen Mary College (salary scale £2,450 by £100 to £3,250 a year plus £80 London Allowance).

Applications (ten copies), must be received not later than November 29, 1965, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF GLASGOW

CHAIR OF PHYSIOLOGY

Applications are invited for appointment to an additional Chair of Physiology for which an Ordinance has been promoted. This is a new appointment.

Applications (20 copies), should be submitted not later than November 26, 1965, to the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

CARDIFF

(PROPOSED WELSH UNIVERSITY OF TECHNOLOGY)

DEPARTMENT OF MATHEMATICS

Applications are invited from Mathematicians and Statisticians for a post as Reader in the above Department and for posts at Senior Lecturer and Lecturer level. Applicants with research interests in any field of mathematics, computing, or statistics will be considered. A Statistician is required for at least one of the posts. Equipment of the Department includes an Elliott 803 Computer. Salary scales: Reader and Senior Lecturer, £2,400 by £100 to £3,000 (or exceptionally to £3,250) Lecturer, £1,400 by £85 to £2,165 by £85 to £2,505.

Further particulars and forms of application (to be returned by November 13, 1965, quoting reference number CN 657), may be obtained from the Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GEOLOGY AND MINERALOGY

Applications are invited for a post of Lecturer or Assistant Lecturer in Geology and Mineralogy. Applicants should have considerable field experience of applied geology, preferably on the side of engineering. Salary on scale: Lecturer, £1,400 to £2,505; Assistant Lecturer, £1,050 to £1,275, all with placing according to qualifications and experience. Superannuation (F.S.S.U.) and removal allowance.

Further particulars should be obtained from the Secretary, the University, Aberdeen, with whom applications (Lecturer 8 copies, Assistant Lecturer 2 copies), should be lodged not later than November 13, 1965.

UNIVERSITY OF SASKATCHEWAN

REGINA CAMPUS

REGINA, SASKATCHEWAN

The Division of Natural Sciences invites applications for the following academic posts as of July 1, 1966. Applicants should have qualified for the Ph.D. degree; and, in the case of senior appointments, have research and teaching experience. Duties to consist of teaching and research at both undergraduate and graduate levels. Completely new laboratories and facilities.

Salary ranges: Professor, $14,000 (12-month basis); Associate Professor, $11,000 to $13,600; Assistant Professor, $8,200 to $10,600.

Rank dependent on training and experience.

Biology—Vertebrate Zoologist, preferably with specialization in vertebrate ecology.

Biochemistry—preferably with specialization in molecular biology.

Inorganic Chemistry—preferably with interest in complex-ion and co-ordination chemistry.

Organic Chemistry—preferably with interest in mechanistic organic chemistry.

Analytical or Physical Chemistry—preferably with interest in spectroscopy.

Geology—field of specialization open.

Mathematics—three positions—one in field of geometry, one in computer science and one open.

Physics—two positions—one in theoretical physics and one open.

Send complete curriculum vitae, small photograph and the names of three or four references or request for further information to: Chairman, Division of Natural Sciences, University of Saskatchewan, Regina Campus, Regina, Saskatchewan.

UNIVERSITY OF CAMBRIDGE, SCHOOL of Agriculture: Applications are invited for the post of University Demonstrator in Agriculture. Candidates should hold an Honours degree and should have postgraduate teaching and/or research experience. Duties: to assist in demonstrating in practical farm and field experimentation classes and possibly some lecturing. Salary £1,200 by £75 to £1,350 by £50 to £1,400 by £85 to £1,570. Age, qualifications and experience will be taken into account in determining commencing salary. A limited contribution may be made toward the removal expenses of the person appointed. The maximum tenure of the post is five years.—Candidates should apply in writing, giving date of birth, details of qualifications and the names and addresses of not more than three referees, to the Secretary, School of Agriculture, Downing Street, Cambridge, not later than November 20, 1965.

UNIVERSITY OF OXFORD. POST-doctoral biochemist required to work on the metabolism of the lens in relation to eye disease. Salary according to experience.—Apply to Dr. R. van Heyningen, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford.

UNIVERSITY OF EDINBURGH

DEPARTMENT OF STATISTICS

SENIOR LECTURERS, LECTURERS AND ASSISTANT LECTURERS

The new Department of Statistics, headed by Professor D. J. Finney, requires staff for 1966-67. A programme of teaching, research and consultation in theoretical statistics and its applications in many fields will be developed as rapidly as possible. The several appointments to be made in 1966 will form the nucleus of a strong Department. Salary scales: Assistant Lecturer, £1,050 by £75 to £1,275; Lecturer, £1,400 by £85 to £1,740; bar, £1,825 by £85 to £2,505; Senior Lecturer, £2,750 by £100 to £3,150 per annum, with placement according to qualifications and experience, and with superannuation benefit.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than December 10, 1965.

CHARLES H. STEWART,
Secretary to the University.

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF ENVIRONMENTAL STUDIES

Applications are invited for an ASSISTANT LECTURESHIP (if experience and qualifications are appropriate a Lectureship would be considered). Candidates should be scientists, preferably qualified in physical geography or in a cognate field. An interest in some aspect of hydrology would be appreciated.

Further particulars may be obtained from the Secretary, University of Lancaster, Bailrigg House, Lancaster, to whom applications should be sent so as to arrive by December 13, 1965.

UNIVERSITY OF GLASGOW

CHAIR OF BIOCHEMISTRY

Applications are invited for appointment to an additional Chair of Biochemistry for which an Ordinance has been promoted. This is a new appointment.

Applications (20 copies), should be submitted not later than November 26, 1965, to the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

UNIVERSITY OF GLASGOW

SENIOR LECTURER IN COMPUTING SCIENCE

Applications are invited for a post of Senior Lecturer in the Computing Department at a salary of £2,400 to £3,150 per annum, from persons with suitable experience in some aspects of Computing Science, such as System Programming.

Applications (eight copies), should be lodged not later than November 15, 1965, with the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

OFFICER REQUIRED BY NATIONAL Vegetable Research Station, Wellesbourne, Warwick, to assist in work on wilt diseases and onion white rot. Qualifications: degree (science, horticulture, or agriculture) or equivalent. Appointment as Assistant Experimental Officer (£719 at 21, rising to £1,201) or Experimental Officer (£1,319 rising to £1,675). Scale and starting point depending upon qualifications, age, and experience. Contributory superannuation scheme.—Applications, with names and addresses of two referees, to Secretary by November 12, 1965.

RESEARCH OPPORTUNITIES

Two openings exist in the Cardiovascular Laboratory of a large drug company's Research Institute which is opening on the outskirts of Toronto.

A. Scientist with Bachelor's or Master's degree; preferably with some experience in animal experimental physiology. Salary range $7,200 to $8,000.

B. Laboratory Assistant with some experience in animal care and experimental procedures. Salary range $4,500 to $5,500.

We are seeking qualified personnel who would like to affiliate with a group dedicated to the efforts of new drug research and development. Our diversified company offers an opportunity for career growth in a world-wide organization.

Applications should be addressed in confidence to:

Warner-Lambert Research Institute of Canada Limited,
727 King Street West,
Toronto 2B, Ontario, Canada.

WESTERN AUSTRALIA

STATE PUBLIC SERVICE
DEPARTMENT OF MINES
GEOLOGICAL SURVEY BRANCH
SUPERVISING GEOLOGIST
(Permanent)

Salary range: £A3,467 (gross) per annum.

Qualifications: University degree with geology as a major subject, with extensive experience in the examination and assessment of mineral deposits, together with administrative ability.

Duties: To supervise and to administer the work of the Mineral Resources Division of the Geological Survey.

Transport: Reasonable amount allowed for transport of appointee with dependent family to Western Australia, provided bond entered into to serve the State for period of three years.

Conditions of service: Superannuation benefits, three months' long service leave after seven years' continuous service, cumulative sick leave entitlements and other general conditions applicable to permanent public servants.

Applications, stating age, qualifications and experience should be addressed by December 3, 1965, to Public Service Commissioner, 184 St. George's Terrace, Perth, Western Australia.

WESTERN AUSTRALIA

STATE PUBLIC SERVICE
DEPARTMENT OF MINES
GEOLOGICAL SURVEY BRANCH
GEOLOGIST, GRADE 2
(Permanent)

Salary range: £A1,572 to £A2,405 (gross) per annum, according to qualifications and experience.

Qualifications: University degree with geology as a major subject. Some experience desirable, but not essential.

Duties: Geological investigations throughout the State.

Transport: Reasonable amount allowed for transport of appointee and dependent family to Western Australia, provided bond entered into to serve the State for period of three years.

Conditions of service: Superannuation benefits, three months' long service leave after seven years' continuous service, cumulative sick leave entitlements and other general conditions applicable to permanent public servants.

Applications, stating age, qualifications and experience should be addressed by December 3, 1965, to Public Service Commissioner, 184, St. George's Terrace, Perth, Western Australia.

POSITION AVAILABLE: RESEARCH Assistant or Associate to aid in the study of the effect of surface condition on crystalline material properties. Should have an interest in low energy electron diffraction.—For further information contact Dr. M. A. Wilkov, Department of Engineering Mechanics, The University of Texas, Austin, Texas, U.S.A.

ST. DUNSTAN'S

A Post-Doctoral Psychologist is required for St. Dunstan's Research Unit at the National Physical Laboratory. The research involved is concerned with matching the sound output of a print recognition device to the human auditory channel and some subjective data using multi dimensional sounds is already available. At the National Physical Laboratory St. Dunstan's is currently evaluating a Sonic Mobility Aid for the blind and the psychological aspects of this project would also concern the successful applicant. The appointment will be for an initial period of two years and the salary will be within the range of £1,200 to £1,850 according to experience.

Applications to the Secretary, St. Dunstan's, 191 Marylebone Road, London, N.W.1.

FIELD STUDIES COUNCIL

Field Assistants (Female) required as soon as possible at Preston Montford Field Centre, near Shrewsbury—applicants should have a degree in Zoology, preferably with an interest in geography or geology; and at Malham Tarn Field Centre, near Settle, Yorks—applicants should have a degree in Geography or Geology and a keen interest in other field subjects and conservation. Salary scale £600 by £30 to £720 per annum with free board and lodging.

Applications, stating age, qualifications, experience and the names of two referees, should be sent to the Secretary, Field Studies Council, 9 Devereux Court, Strand, London, W.C.2.

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE

INVERGOWRIE, DUNDEE

Officer to pursue research in control of weeds in horticultural crops. Salary in Scientific Officer (£895 to £1,521) or Senior Scientific Officer grade (£1,685 to £2,082); F.S.S.U.

Further details may be had from the Secretary, Scottish Horticultural Research Institute, Invergowrie, Dundee, to whom applications, supported by the names of two referees, should be sent by December 15.

WEST OF SCOTLAND AGRICULTURAL COLLEGE

Applications are invited for a post of Lecturer in Bacteriology from candidates with a degree in Bacteriology or equivalent qualification. The salary will be (according to age, qualifications and experience) on one or other of the following scales: Grade III, £1,349 rising to £2,119; Grade IV, £869 (Honours £895) rising to £1,234.

Conditions of appointment and application forms are obtainable from the Secretary, The West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2, with whom applications should be lodged not later than November 15, 1965.

UNIVERSITY OF GUELPH

ONTARIO AGRICULTURAL COLLEGE
GUELPH, CANADA
DEPARTMENT OF NUTRITION

Applications are invited for the position of Scientist (salary range for Bachelor's degree, $5,250 to $6,300 or Master's degree, $6,300 to $7,500). Candidates should have a strong background in chemistry for biochemical research in metabolism and nutrition of mammals and birds.

Applications should be addressed to Dr. S. J. Slinger, Head, Department of Nutrition.

APPLICATIONS ARE INVITED FOR Research posts in the following subjects: Primatology, Comparative Osteology and Palaeolithic Prehistory. Applicants should have University degree in appropriate subjects and application for study of primate disease by medical or veterinary candidates would be considered. Interviews will be arranged London last week November.—Apply by airmail, Dr. Leakey, P.O. Box 30239, Nairobi, Kenya.

UNIVERSITY OF CAMBRIDGE, PHYSIOlogical Laboratory, invites applications for new Senior Animal Technician's post in Higher Technical grade. Salary scale, £985 to £1,362 per annum. Extensive scientific or agricultural experience with animals essential; I.A.T. membership desirable.—Written applications, giving age, experience, qualifications to Superintendent, Physiological Laboratory, Downing Street, Cambridge.

JUNIOR TECHNICAL OFFICER, EXPERIenced, with biochemical or microbiological background, capable of working without supervision (graduate with pass degree preferred) required for varied and interesting work in a biological research laboratory moving to Edinburgh University in eighteen months. Salary on Medical Research Council scale and commensurate with age and experience.—Apply to The Director, Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W.12.

NEW ZEALAND

MARINE DEPARTMENT

Applications are invited for the undermentioned vacancy:

Vacancy B 13/13/9/4160
FISHERIES SCIENTIST

Salary: Up to £1,825 according to qualifications and experience, with prospects of further promotion on merit.

Qualifications desired: Degree in Science preferably Master of Science and experience of management of shell-fish population.

Duties: Supervision of the operation of and management of the Foveaux Strait oyster fishery which includes sampling and measurement of the catch and effort on various designated beds.

Passages: Fares for appointee and his wife and family, if married, will be paid.

Incidental expenses: Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, with whom applications will close on December 3, 1965.

Please quote reference B 13/13/9/4160 when inquiring.

NEW ZEALAND

ELECTRON MICROSCOPIST

APPLICATIONS are invited for an ELECTRON MICROSCOPIST to join a Research Group in the Meat Industry Research Institute of New Zealand (Inc.), working in the fields of muscle metabolism and structure.

The Institute is the central research organization for the New Zealand Meat Industry, and undertakes both applied and basic investigations. The Electron Microscopist will be encouraged to initiate and conduct original investigations within an active academic and strong research environment. He will also be expected to give advice and help to the adjoining Ruakura Agricultural Research Centre (animals and soil research) of the Department of Agriculture.

The Institute's laboratories are spacious, well-equipped, and with excellent library facilities. The electron microscope (Philips E.M. 200) is housed in a newly designed suite.

Applicants are required to have an honours degree or equivalent qualification, with experience in the use of the electron microscope, preferably in biological research.

A Superannuation scheme operates, and professional officers are given the opportunity at intervals for overseas study leave.

Passage: Fares for the appointee (and his wife and family, if married) will be paid.

Incidental Expenses: Up to £35 for a single man, and £100 for a married man, can be claimed to cover the cost of taking personal effects to New Zealand.

Salary Range: £1,800 to £2,500 according to qualifications and experience. Prospects of advancement are excellent and will be based on scientific merit.

Further particulars may be obtained from either—The Director, Meat Industry Research Institute of New Zealand (Inc.), P.O. Box 617, Hamilton, New Zealand, or—The Senior Scientific Liaison Officer, New Zealand Scientific Office, Africa House, B.C.S.O., Kingsway, London, England.

Applications, with names of two referees, to be sent (airmail) to either—The Director, Meat Industry Research Institute of New Zealand (Inc.), P.O. Box 617, Hamilton, New Zealand, or—The Senior Scientific Liaison Officer, New Zealand Scientific Office, Africa House, B.C.S.O., Kingsway, London, England.

APPLICATIONS will CLOSE on January 31, 1966.

MOREDUN INSTITUTE

EDINBURGH

Assistant Experimental Officer/Experimental Officer, Experimental Animal Unit. Over 21 with pass degree or equivalent qualification. Some experience in microbiology and interest in animal breeding essential. There will be opportunity for research. Salary £719 to £1,201 (A.E.O.), £1,319 to £1,675 (E.O.), according to age and experience.

Applications, in writing, with the names of two referees to the Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh, 9.

UNIVERSITY OF LEICESTER

GENERAL PHYSIOLOGY

Applications are invited for (a) a Senior Lectureship and (b) a Lectureship in the new Laboratory of General Physiology. Salary scales: Senior Lecturer, £2,590 to £3,250; Lecturer, £1,400 to £2,505; initial salary according to qualifications and experience.

Further particulars from the Registrar, to whom applications should be sent by November 17.

CHIEF ELECTRONIC ENGINEER wanted. Degree or H.N.C. standard required Extensive experience of latest transistor techniques absolutely necessary. Duties will include organization of electronic department and research in various fields, including proton and other precession magnetometers, potentiometric recorders, analytical equipment, etc. Also some testing of special equipment will be included. An excellent opportunity for the enthusiastic man. Salary up to £1,800 per annum, but will depend on suitability.—Reply to Dr. E. T. Hall, Littlemore Scientific Eng. Co., Littlemore, Oxford.

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY

RUTHERFORD SCHOLARSHIP

Applications are invited from graduates of a university within the British Commonwealth who are under 26 years of age, or if holding the degree of Ph.D. under 28 years of age, on May 1, 1966, for the RUTHERFORD SCHOLARSHIP. This will be awarded for experimental research in any branch of the natural sciences, but if there are candidates of similar merit preference will be given to experimental physics. The scholarship will be tenable for three years in some part of the British Commonwealth other than that in which the applicant graduated. It will not normally be awarded to a graduate who has already held a senior research award.

The value of the scholarship will be adjusted to meet the circumstances of the appointment but if held in the United Kingdom it will be between £850 and £1,050 per annum (or up to £1,250 per annum for post-doctoral candidates). Additional allowances will be granted for travel, university fees, etc. The appointment will date from October 1, 1966, or such other date as may be arranged.

The appointment will be made by the President and Council of the Royal Society. Application from university graduates outside the United Kingdom should be made through their universities to the Royal Commission for the Exhibition of 1851 on forms of application for the 1851 Exhibition Science Research Scholarship. Application from university graduates in the United Kingdom should be made before February 15, 1966, on forms obtainable from the Executive Secretary, The Royal Society, Burlington House, London, W.1.

COMMONWEALTH SCHOLARSHIP AND FELLOWSHIP PLAN

AWARDS BY CEYLON AND INDIA

Applications are invited for Commonwealth Scholarships tenable in:

(1) Ceylon at the University of Ceylon, Vidyadaya University, Vidyalankara University, and the Ceylon Technical College from June/July, 1966.

(2) India at Indian universities and institutions of higher learning from June/July, 1966.

Commonwealth Scholarships are in general tenable for a programme of study or research normally extending over a period of two academic years and leading to a higher degree or similar qualification. The awards are normally open to men and women students under the age of 35 whose permanent homes are in Britain and who are citizens of the United Kingdom or some other Commonwealth country. Emoluments will cover fares, fees, and maintenance allowances of up to Rs.800 to Rs.900 per month. Certain other allowances are also payable.

Further particulars and application forms may be obtained from the Secretary-General, The Association of Commonwealth Universities, Marlborough House, Pall Mall, London, S.W.1, with whom applications must be lodged not later than November 15, 1965.

LOUGHBOROUGH COLLEGE OF TECHNOLOGY

LOUGHBOROUGH, LEICESTERSHIRE

(UNIVERSITY DESIGNATE)

DEPARTMENT OF ELECTRICAL ENGINEERING

Applications are invited from Graduate Electrical Engineers and Physicists for a Research Scholarship, tenable in the above Department, for work on the design of certain types of reflectors for aerial arrays. The value of the Scholarship will be between £400 and £525 per annum.

Further particulars and application form are available from the Academic Registrar, quoting reference 55/AF.

FELLOWSHIPS AND STUDENTSHIPS

BEIT FELLOWSHIP FOR SCIENTIFIC RESEARCH

Notice is hereby given that the forty-eighth Election will take place in 1966. Applications must be received on or before January 1, 1966, and candidates must be under the age of 25 at the date of election.

Forms of application and all information may be obtained by letter addressed to the Registrar, Imperial College, South Kensington, London, S.W.7.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF COAL RESEARCH

POST-DOCTORAL FELLOWSHIPS IN COMBUSTION RESEARCH

Applications are invited from Physical Chemists, Physicist and Spectroscopists for appointment to Postdoctoral Fellowships tenable with the Organization's Division of Coal Research, located at North Ryde, Sydney, N.S.W.

A major part of investigations undertaken by the Division of Coal Research concern the solution of industrial problems involved in the utilization of solid fuels in the power generation and metallurgical industries. To facilitate these investigations, the Division is expanding its research activities in the field of basic combustion science. Current research includes investigations in combustion kinetics, surface chemistry, flame chemistry and high-temperature inorganic chemistry.

The Division is very well equipped and the facilities available include those for optical spectroscopy from ultra-violet to the far infra-red, electron spin and nuclear magnetic resonance spectroscopy, electron and optical microscopy, electron and x-ray diffraction, mass spectrometry and the measurement of surface properties.

Successful applicants would be required to work on one or other of the following topics:—

1. The kinetics of rapid combustion of fine particles.
2. The kinetics of atomic and free-radical reactions in the gaseous phase.
3. Factors affecting the electrical properties of inorganic solids of low conductivity with particular reference to surface properties.
4. Mass spectrometric studies of the chemistry of inorganic additives in flames.
5. Application of infra-red spectroscopic techniques to the study of high-temperature chemistry of inorganic substances.

Applicants should have a Ph.D. degree with basic training in Physics or Physical Chemistry or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

SALARY: Dependent upon qualifications and experience within the ranges of:—

£A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum.

Salary rates for women are £A201 per annum less than the corresponding rates for men.

The Fellowships will initially be for a fixed term of three years. Consideration will be given to the continuation of F.S.S.U. arrangements.

Fares paid for the appointee and his dependent family and return fares to point of origin will be paid at the end of the appointment. Further particulars supplied on application to:

Mr. W. Hartley, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.,

to whom applications (quoting Appointment No.: 480/496) should be addressed by December 11, 1965.

MEDICAL RESEARCH COUNCIL

TRAVELLING FELLOWSHIPS IN MEDICINE AND MEDICAL SCIENCE

The Medical Research Council invite applications for the following Travelling Fellowships for the academic year 1966-67.

ALEXANDER PIGOTT WERNHER MEMORIAL TRAVELLING FELLOWSHIPS IN OPHTHALMOLOGY AND OTOLOGY

These Fellowships are intended for suitably qualified medical graduates who are likely to profit by a period of work in Ophthalmology or Otology in the United Kingdom or at a centre abroad before taking up higher teaching and/or research posts in these branches of medical science. Candidates will be required to submit an outline of their proposals for work at a selected centre. The awards will normally be tenable for one year, but grants may also be given for short-term visits abroad to study new methods of investigation and treatment in these fields.

Fellowships tenable in the United Kingdom will carry a stipend within the range of £900 per annum to £1,120 per annum depending on the candidate's age and experience; in addition, married men will receive a dependants' allowance of £250 per annum. Fellowships tenable in the United States will carry a stipend within the range of $5,000 per annum to $6,000 per annum depending on the candidate's age and experience and where appropriate an allowance of $500 per annum will be payable for the fellow's wife and each dependent child. In addition, an allowance will be made for travelling and other approved expenses.

Further particulars of these awards and forms of application may be obtained from the Secretary, Medical Research Council, 20 Park Crescent, London, W.1, to whom completed forms should be returned by not later than November 30, 1965.

ROYAL NAVAL COLLEGE

GREENWICH

RESEARCH FELLOWSHIP

Applications are invited for a Junior Research Fellowship in the Metallurgy Department to undertake research (supported by D.S.I.R.) in the field of brittle fracture of structural steels and the effect on this of fatigue loading. Candidates should have graduated in metallurgy and have a good knowledge of steels. Fellowship is tenable for three years at a fixed salary within the range £975 to £1,310. F.S.S.U. provision.

Fuller details of the proposed field of research can be obtained from Professor A. G. Dowson, R.N. College, Greenwich, S.E.10.

POSTDOCTORAL FELLOWSHIP ($6,000 tax-free stipend) in protein chemistry available early 1966 for applicants with previous experience in this area. Study will involve physicochemical, chemical and enzymatic investigations. —Apply with curriculum vitae and two letters of reference to Dr. C. M. Kay, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

C.S.I.R.O. — AUSTRALIA
DIVISION OF ORGANIC CHEMISTRY

FELLOWSHIPS AND RESEARCH APPOINTMENTS

ORGANOMETALLIC CHEMISTRY

exchange reactions of Al and Zn compounds
new organic derivatives of Zr and Ti

NATURAL PRODUCTS CHEMISTRY

isolation and structure determination of biologically active substances of medicinal interest, mainly alkaloids

substances toxic to grazing animals

GENERAL SYNTHETIC ORGANIC CHEMISTRY

new products and processes, especially in relation to the organometallic and natural products programme

The Division of Organic Chemistry is one of the constituent Divisions of the Chemical Research Laboratories. It is well equipped with modern facilities including an automatic scintillation counter and N.M.R. and mass spectrometry.

Qualifications: Applicants should have a Ph.D. degree in Chemistry, or have had appropriate postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

Fellowships: Available on a two or three year basis with fares, where applicable, to and from Australia, for the appointee and his wife and dependent family.

Salary: £A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum, dependent on qualifications and experience.

Research Appointments: Eligible for superannuation, subject to medical examination. Fares for appointee and his wife and dependent family to Australia. Further particulars in respect of removal allowances may be obtained on request. A probationary period may be specified initially.

Salary: £A2,445 to £A3,105 per annum or £A3,235 to £A3,755 per annum, dependent upon qualifications and experience. For an outstanding scientist with a record of research leadership, a range of £A3,885 to £A4,485 per annum would be considered.

Note: All salaries quoted are for men; women are paid £A201 per annum less.

Applications (quoting Appointment No. 606/70 and stating full name, place, date and year of birth, nationality, marital state, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing) should reach:

Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
Africa House,
Kingsway,
London, W.C.2, England,

by December 18, 1965.

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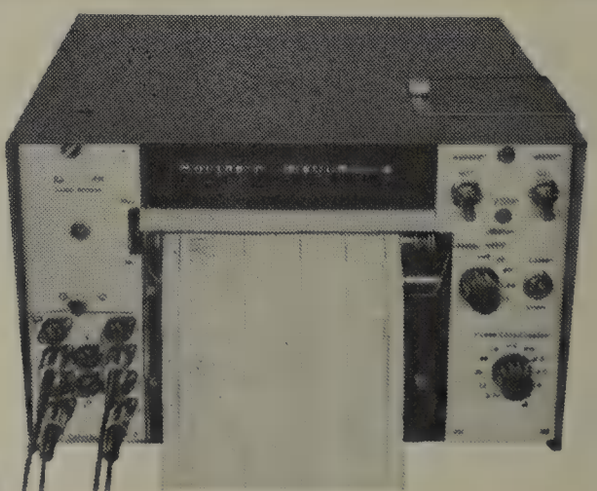

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THE CIBA FELLOWSHIP TRUST

Opportunities for scientific research on the Continent of Europe

Applications are invited for a number of CIBA Fellowships which will be awarded in March 1966, to enable graduates of Universities in the United Kingdom and the Republic of Ireland to carry out research at Continental Universities in chemistry, physics or some other allied scientific subjects. Applicants may be at present either at University or in Industry.

The basic values of the Fellowships are £700 for pre-doctoral and £1,100 for post-doctoral Fellows. Additional allowances are made for travel to and from the Continent and according to marital status. Fellowships are tenable for one year in the first instance but there are opportunities for renewal for a further one or two years.

The CIBA Fellowship Trust was founded in 1958 by the CIBA Companies of Great Britain for the purpose of improving and increasing the interchange of ideas between scientists in the United Kingdom and the Continent. Those interested should write to the Secretary, The CIBA Fellowship Trust, 96 Piccadilly, London W.1, who will provide full details and application forms.

The closing date for applications is 14th January 1966.

CIBA

UNIVERSITY OF SOUTHAMPTON

RESEARCH FELLOW ON HOVERCRAFT

Applications are invited from suitably qualified graduates for the post of Research Fellow in the Department of Aeronautics and Astronautics for work in Fluid Mechanics applied to Hovercraft. The salary will be based on the qualifications and previous experience of the candidate but would be in the region of £1,200 per annum.

Applications (in duplicate), giving details of qualifications and experience, together with the names of two referees, should be sent to the Deputy Secretary, The University, Southampton, before November 15, 1965.

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WANTED. SECOND-HAND UNICAM Spectrophotometer SP500.—If you have one for disposal please write to the Departmental Superintendent, Department of Chemistry, Imperial College, London, S.W.7.

LECTURES AND COURSES

UNIVERSITY COLLEGE LONDON (Gower Street, W.C.1). An Inaugural Lecture by Dr. R. G. Hopkinson, Haden-Pilkington Professor of Environmental Design and Engineering, entitled "The Evaluation of the Built Environment" will be given on Thursday, November 11 at 5.30 p.m. Admission free, without ticket.

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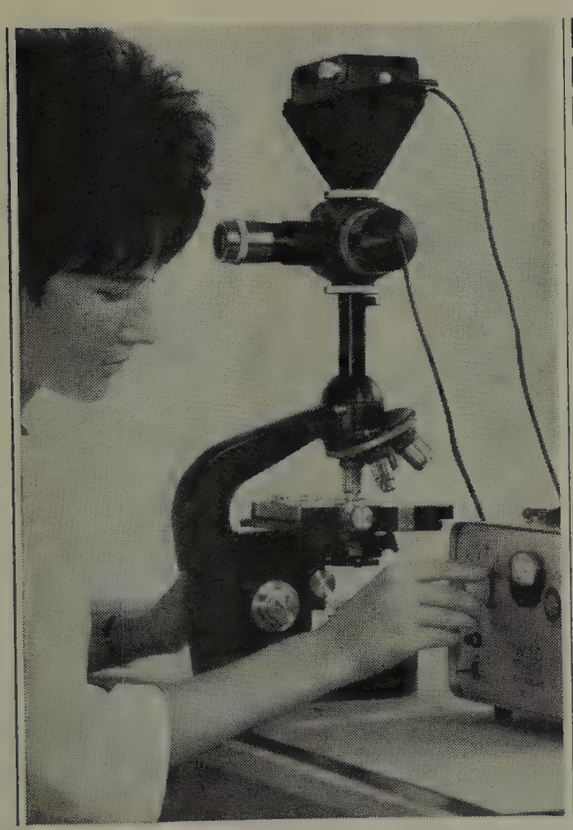

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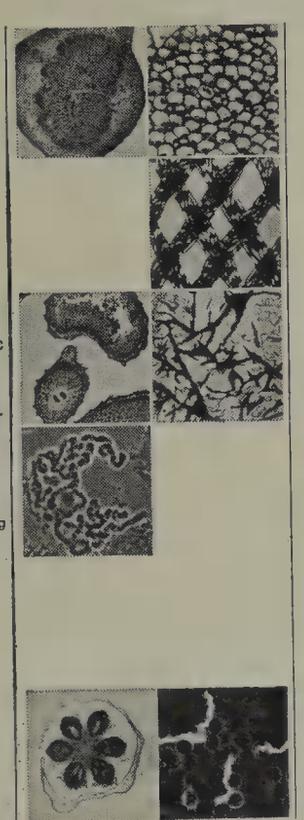

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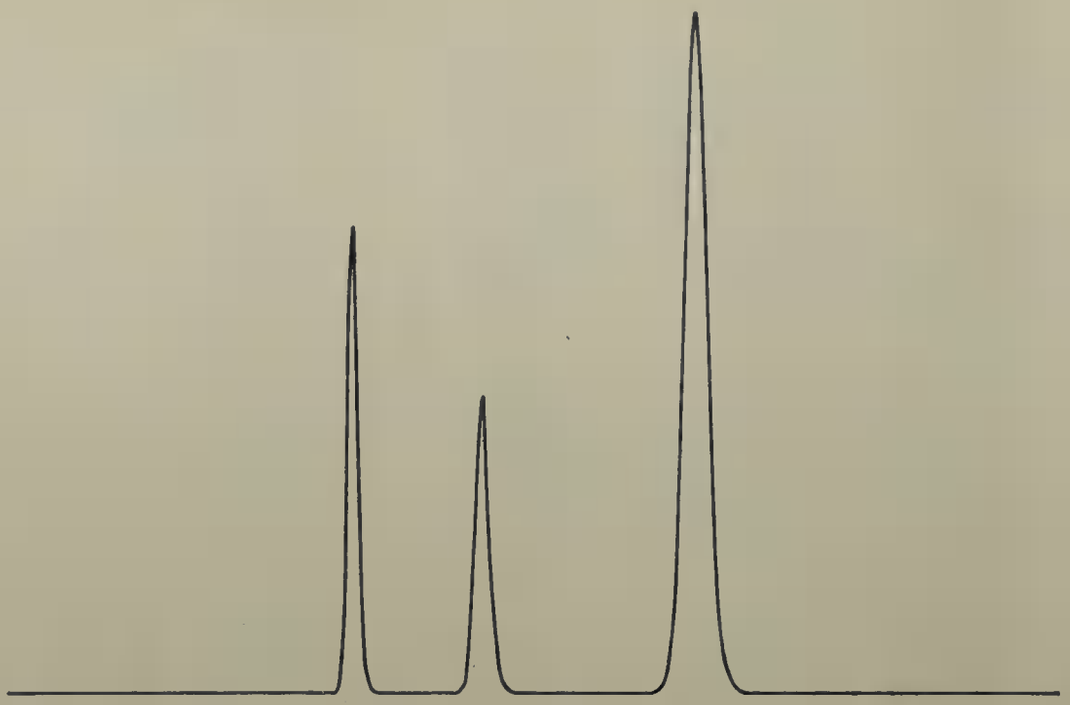

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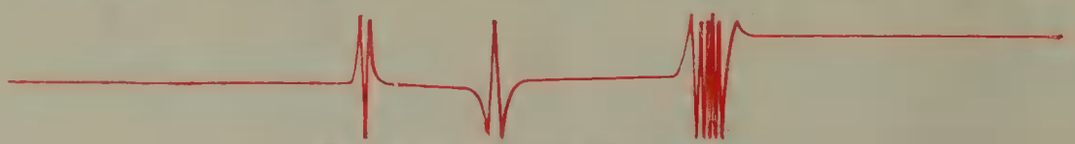

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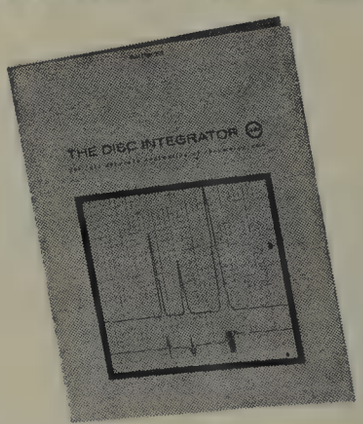

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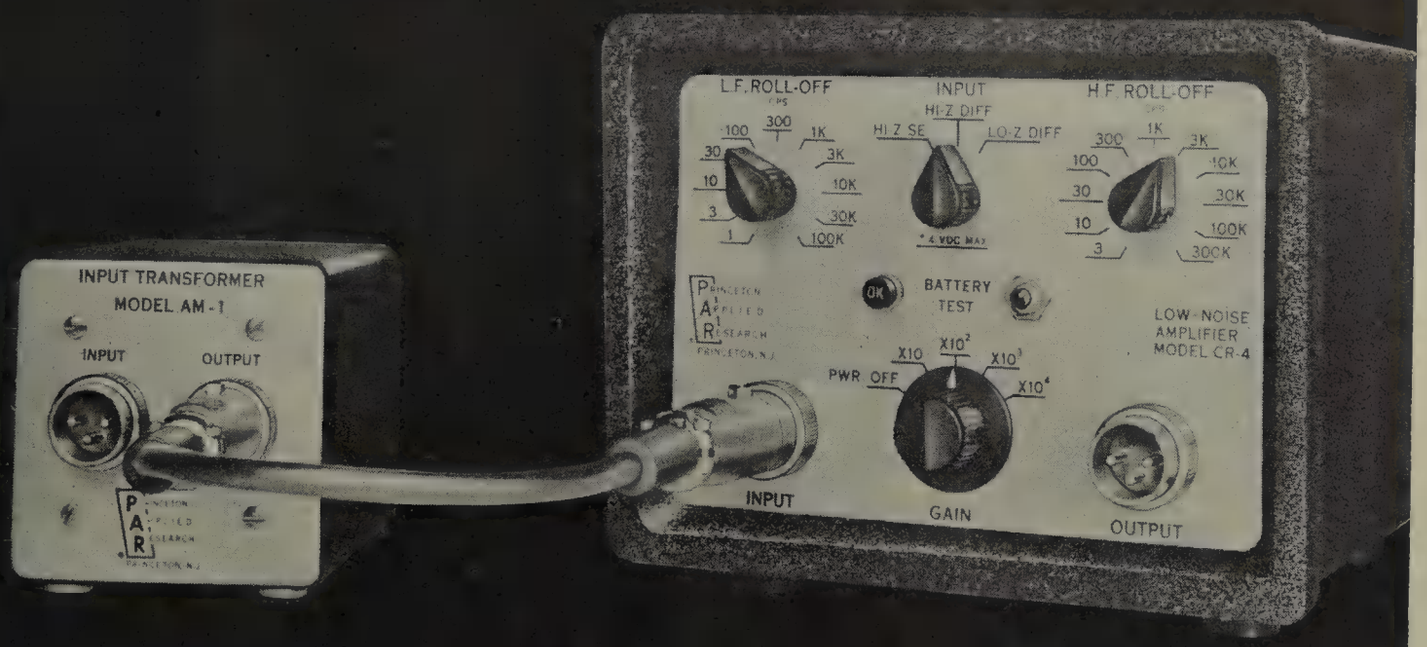

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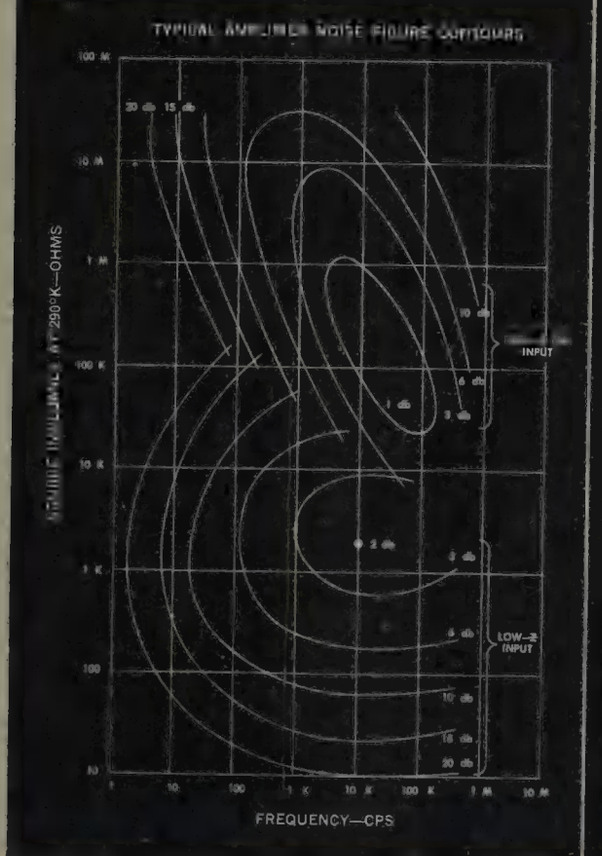

NOISE FIGURE CONTOURS

Experiments in general are performed at some definite source impedance level and over some definite frequency bandwidth. Noise referred to shorted input as frequently specified does not provide meaningful information for actual experimental applications. It is instead essential to know the noise characteristics of an amplifier not only at the particular operating frequencies but also with the particular source impedances actually encountered. Noise figure contours are the loci of points of constant noise figure (F) plotted as a function of source impedance and operating frequency, and, therefore, provide this complete information. They indicate the degradation in signal-to-noise ratio obtained with the pre-amplifier at a particular frequency with a particular source impedance, input noise being the Johnson noise generated by that source impedance at 290 degrees Kelvin. The contours are obtained from the following equation:

$$F = 20 \log_{10} \frac{\text{total measured amplifier noise (including Johnson noise)}}{\text{calculated Johnson noise}} \text{ DB}$$

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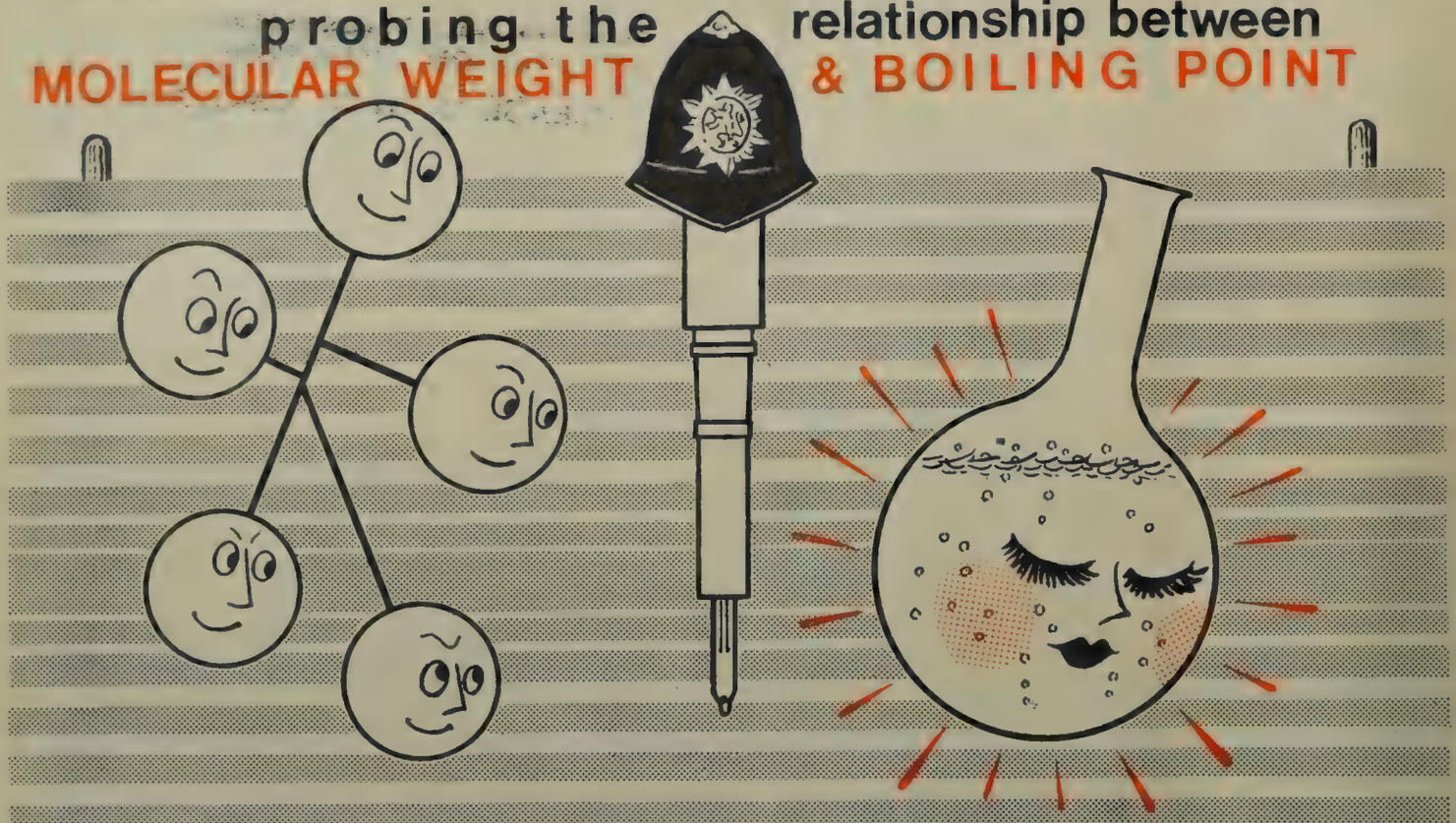

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(1) E. Gurr (1965), Rational Use of Dyes in Biology & General Staining Methods. (Leonard Hill (Books), London. Williams & Wilkins, Baltimore.)

(2) M. A. MacConaill & E. Gurr (1964), *Irish J. Med. Sc.* (June).

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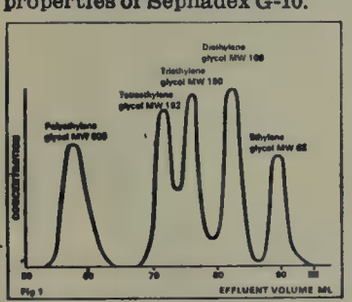

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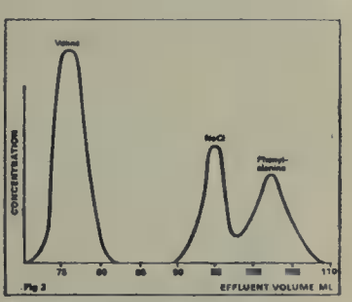

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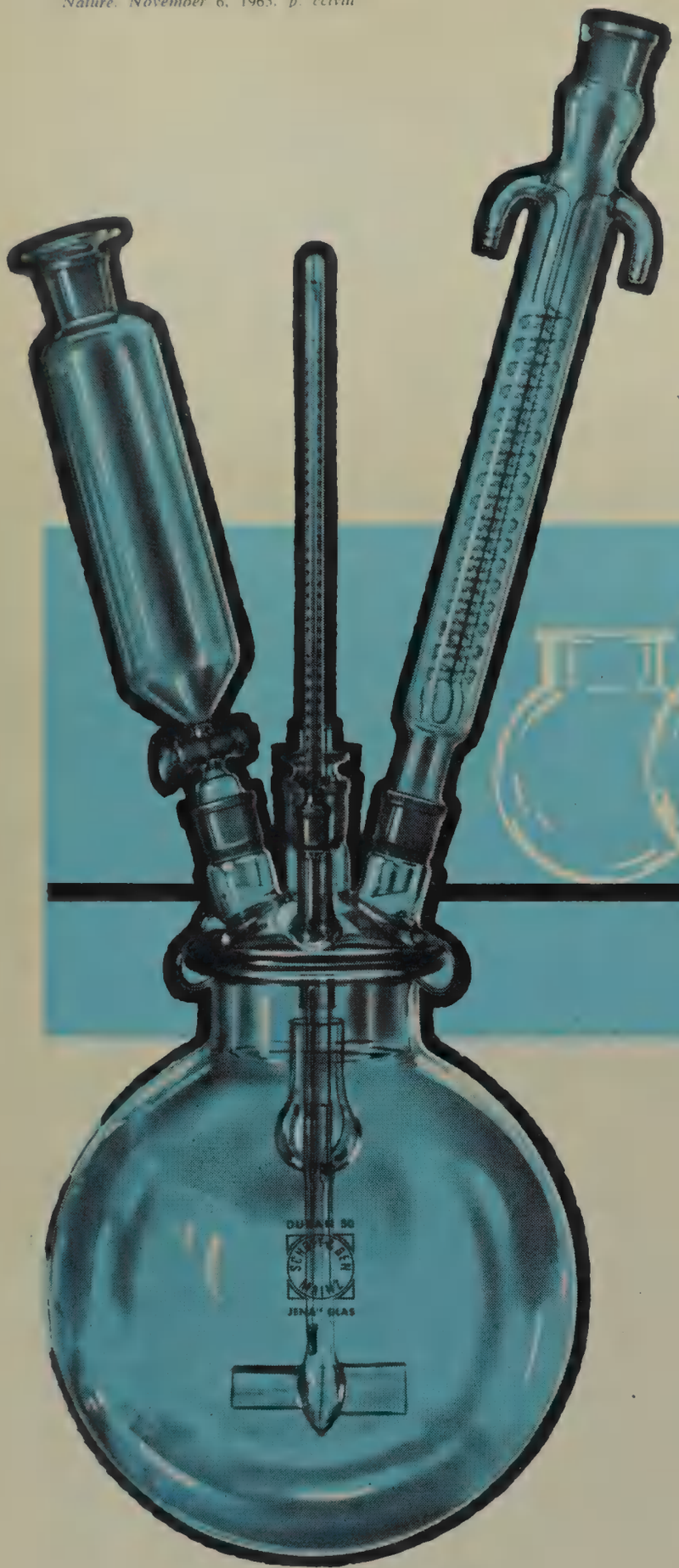

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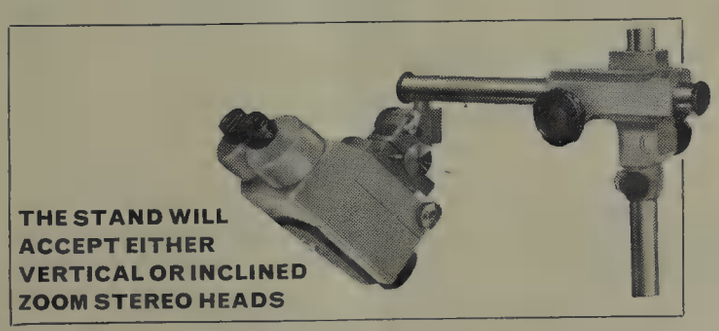

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BULLETIN NO. 5

MOLECULAR STRUCTURE DETERMINATION WITH THE AEI MS9 MASS SPECTROMETER

Metastable Peaks in Mass Spectra provide invaluable additional information on Structure

The mass spectrometer is one of the most powerful instruments for providing identification and structural information for organic compounds. Not only does it give the molecular weight directly (Fig. 1) but modern high resolution instruments such as the A.E.I. MS9 give the molecular composition directly as well. This is not all—fragment peaks indicate the ready loss of structurally significant groups in the molecule.

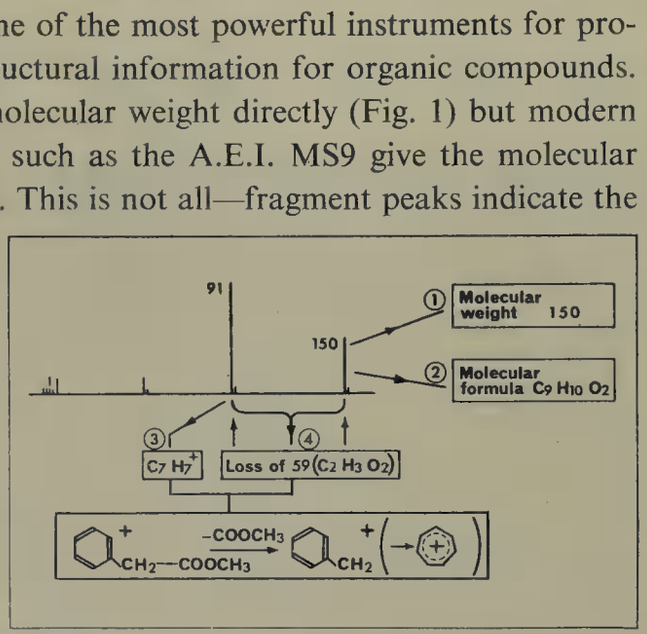

FIG. 1

It is often important to know the mechanism by which a fragment is formed. Where several possibilities exist, the presence of the so-called *metastable* peaks can provide confirmation of a postulated process. Thus in dihydrorobustic acid, the peak at mass 235 is known by mass measurement to be $C_{13}H_{15}O_4^+$. The presence of a metastable peak at mass 144.6 corresponding to the process $C_{22}H_{22}O_6^+ \rightarrow C_{13}H_{15}O_4^+ + C_9H_7O_2$ indicates that a $C_9H_7O_2$ unit can be lost in one step and therefore exists as a structural unit in the molecule. Other possibilities for the formation of the 235 peak, such as the loss of CH_3O followed by the loss of another group C_8H_4O, are thus ruled out.

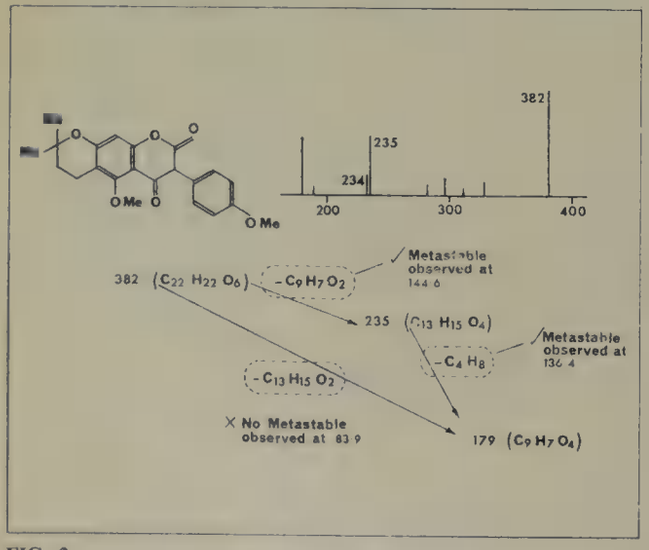

FIG. 2

On the other hand, the 179 peak ($C_9H_7O_4^+$) is shown (Fig. 2) to be due to the loss of a C_4H_8 entity from the 235 peak, not to loss of $C_{13}H_{15}O_2$ from the parent peak.

It is clear that for maximum structural information we shall want to observe intense and well-defined metastable peaks in a mass spectrum.

What is a metastable peak?

Fragmentation normally takes place in the ion source by unimolecular decomposition of the parent ion m_1^+. This gives rise to fragment ions m_2^+ from processes such as $m_1^+ \rightarrow m_2^+ + m_3$. Ion m_2^+ is observed but there is no direct evidence as to how it is formed (Fig. 3).

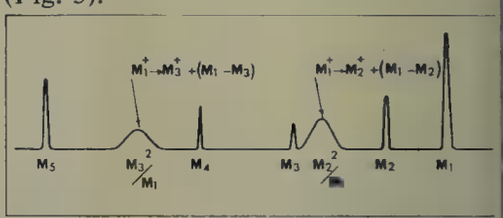

FIG. 3

But this ion decomposition process can occur also during the flight of the ion through the mass spectrometer. If it occurs within a certain region of the ion path, the resulting m_2^+ ion reaches the collector but gives rise to a metastable peak at an apparent mass m_2^2/m_1. In the MS9 the normally observed transitions occur along the portion 'cd' of the ion path. See Fig. 4.

Why Mass m_2^2/m_1?

The resultant ion m_2^+ has mass m_2, but its energy is not the normal energy eVa but $\frac{m_2}{m_1} \times$ eVa. The apparent mass m* at which the ions appear is therefore not m_2 but $\frac{m_2}{m_1} \times m_2 = m_2^2/m_1$. This apparent mass is not usually a whole number.

Why are they broad?

The broad diffuse shape is explained by the release of internal energy in the fragmentation process. The kinetic energy imparted to the resultant ion m_2^+ means that the ions are no longer sharply focused, and a broad peak results. This however, serves as an easy means of distinguishing between normal and metastable peaks.

Furthermore, for those interested in the energetics of these ion decomposition processes, the width of the peak can be used to determine the internal energy released.

What are the best conditions for observation?

For maximum usefulness metastable peaks should be of reasonable intensity and well-defined apparent mass; they should be easy to distinguish from the normal peaks in the spectrum. The factors involved have been considered in detail in a paper presented at the ASTM E-14 meeting in 1964.†

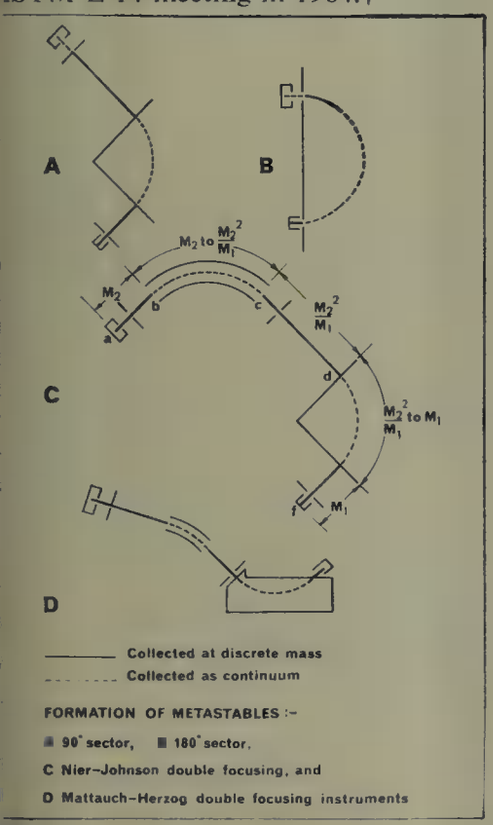

FIG. 4

The intensity is a function of the length of path over which observable transitions can take place. In the MS9 (C) the path 'cd' is long, and since the resultant diverging ions are partially focused, intense metastable peaks are observed. Much lower intensities are found with the Mattauch-Herzog analyser system (D) where focusing is much less effective and where path 'cd' is often shorter (Fig. 4). The Nier-Johnson ion optical system of the MS9 is such that the position of the metastable peaks corresponds accurately with the expected m_2^2/m_1, in contrast with the 180° analyser system (B) where the peaks can be significantly displaced.

The collector slit width plays a major role in determining intensity and observability of metastable peaks. This is illustrated in the spectra shown in Fig. 5 where resolution is varied by altering the collector slit only. At high resolution the intensity of the metastable peaks is far too low for practical use. On the other hand, at low resolution the metastable peaks are intense, but are rather difficult to distinguish from the normal peaks. The optimum condition is evidently a resolution in the region of 1000, where the intensity is still reasonable but the normal and metastable peaks appear as two distinct and easily distinguishable sets.

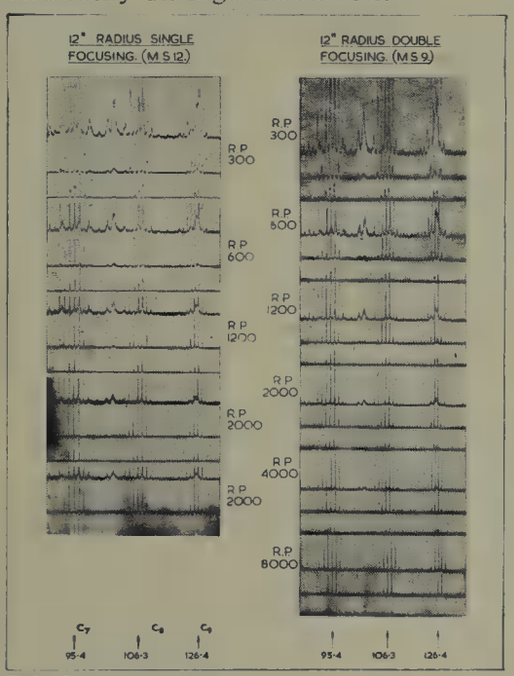

FIG. 5

Ideal Instrument

Thus for metastables to be readily observed under optimum conditions, the ideal instrument should be double focusing so that a resolution of 1000 is readily obtained, but should have an easily adjustable collector slit, so that operation is not restricted to high resolution conditions. The MS9 fulfils both these requirements.

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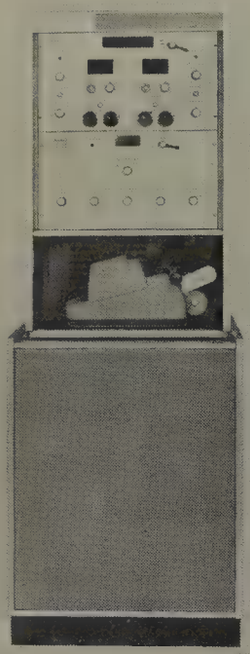

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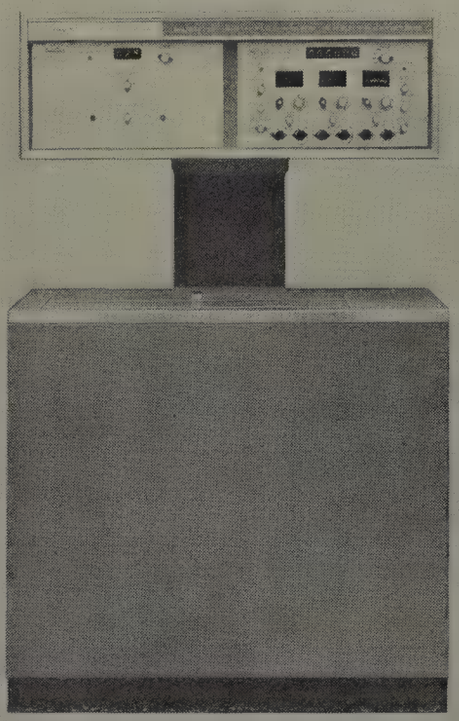

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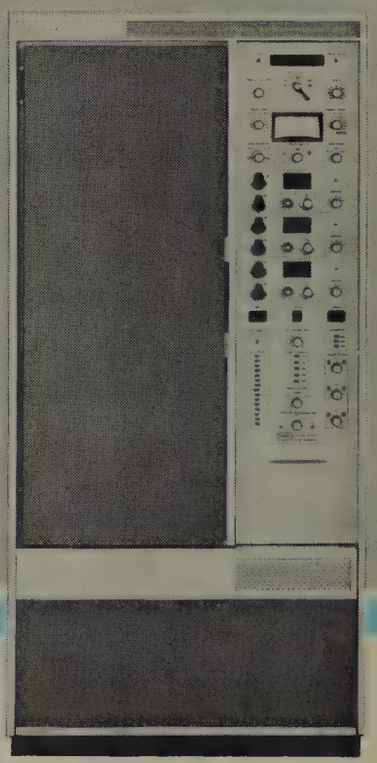

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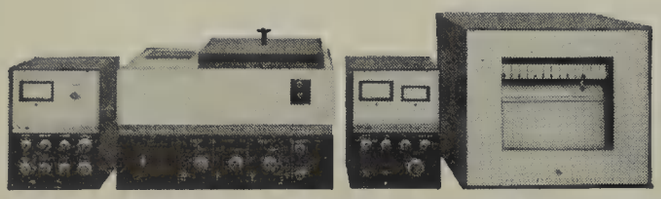

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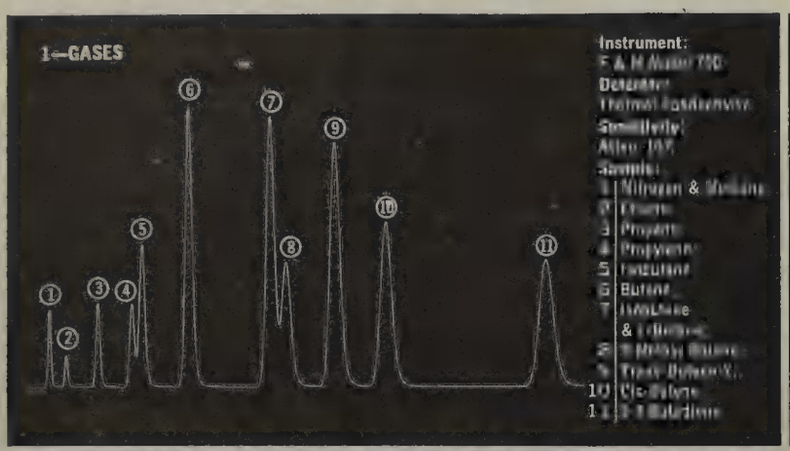

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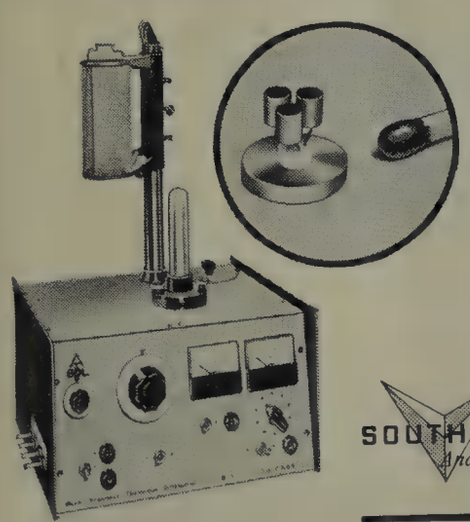

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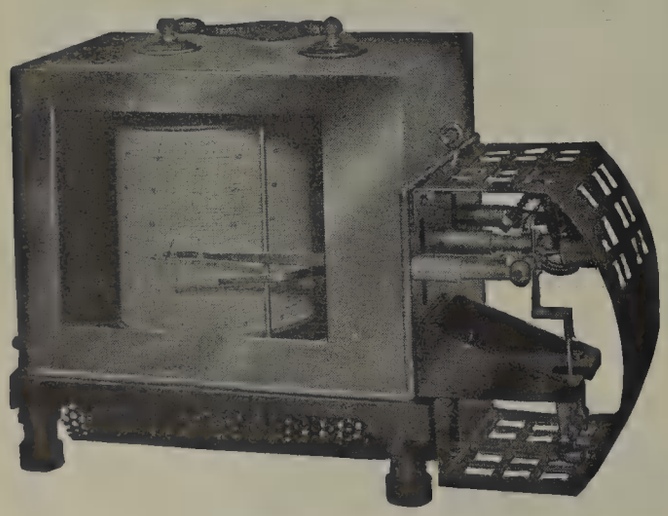

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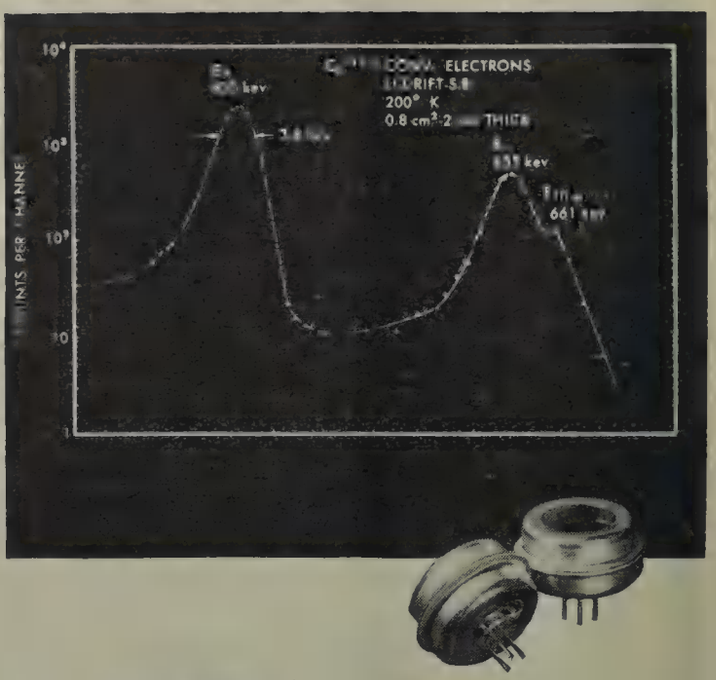

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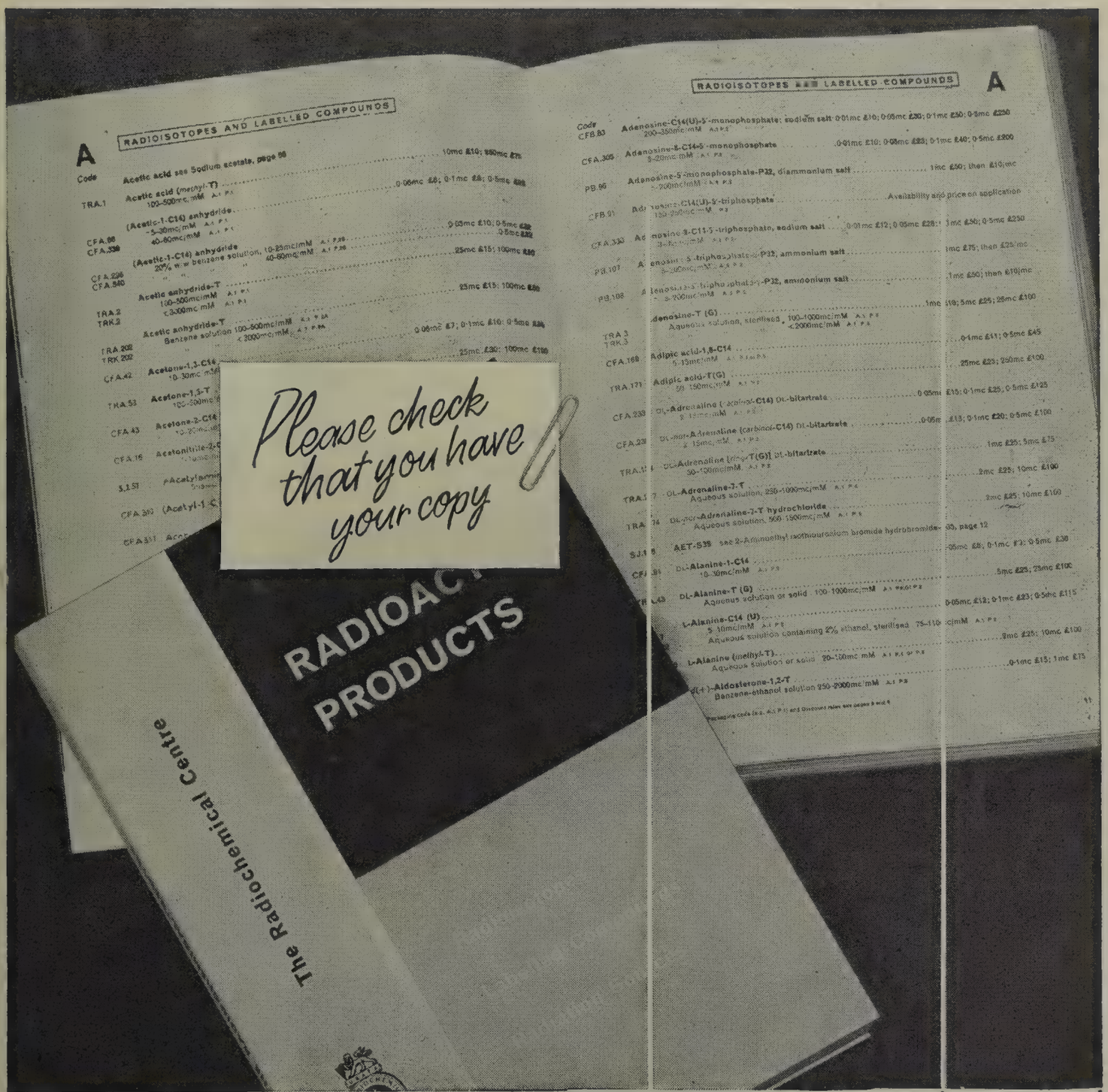

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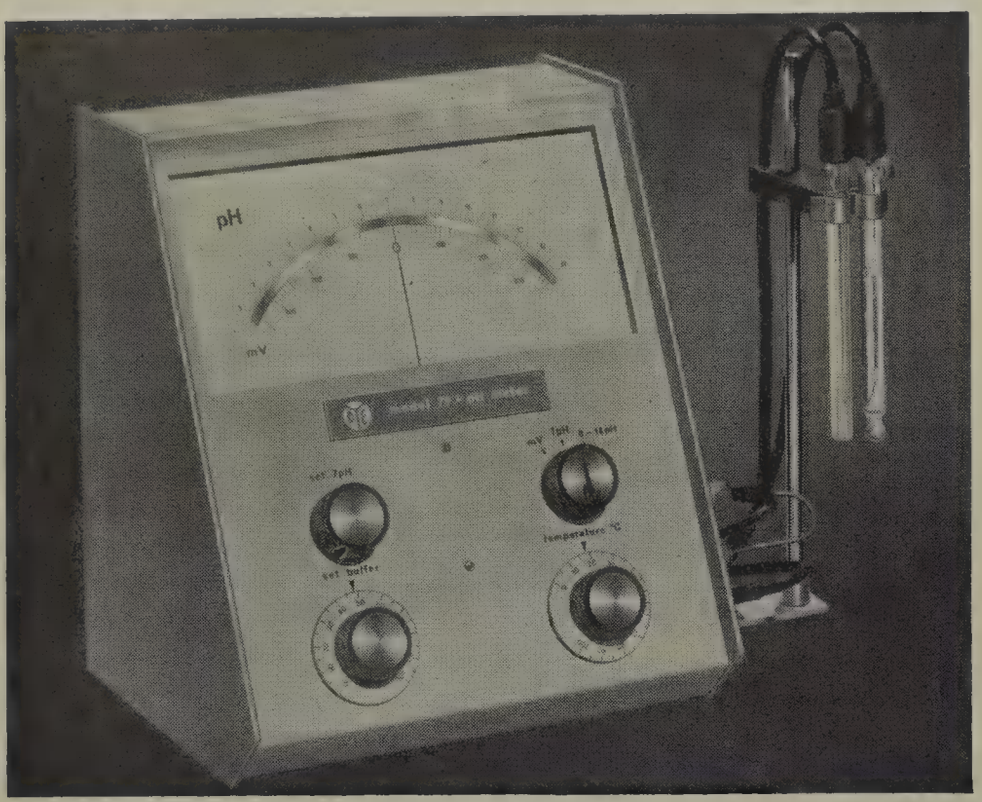

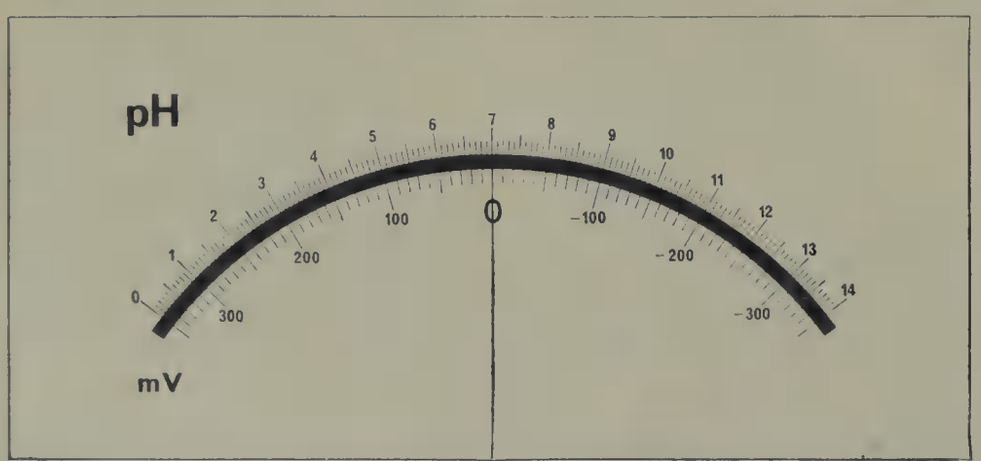

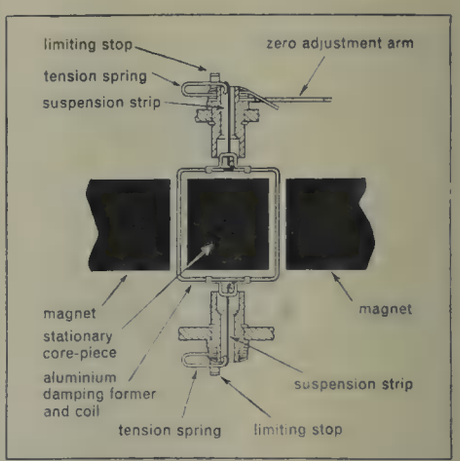

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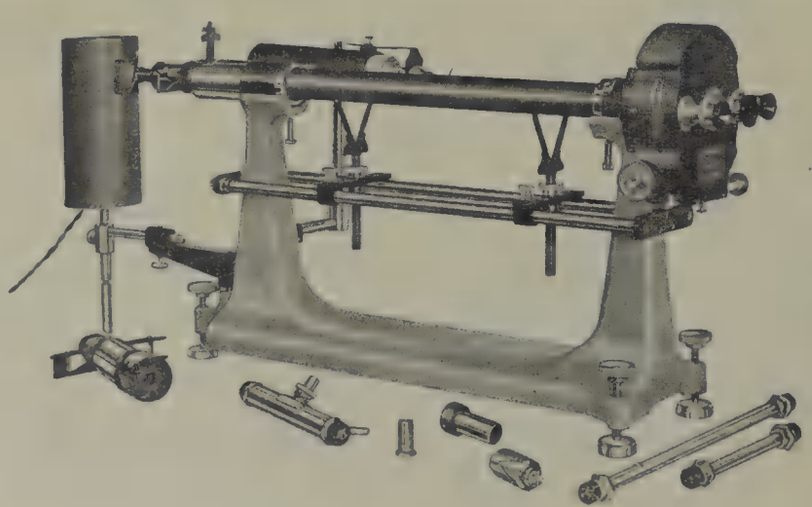

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THE ROAD TO PEACE

IT is now more than twenty years since the United Nations Charter was signed on June 26, 1945, at the San Francisco Conference, but although the anniversary was duly observed at San Francisco the dominant note was gloom rather than rejoicing. The problem of paying for past and future operations of the United Nations was still unsolved and was overshadowed by the continuing clash over Vietnam. Moreover, when on October 4 Pope Paul addressed the General Assembly of the United Nations, while neither of these had been resolved, the conflict in Kashmir had added to immediate anxieties.

Little wonder is it that there should be deep concern over the future of the United Nations, quite apart from any disappointment over failure to fulfil the high hopes entertained twenty years ago. While it is indeed appropriate that we should re-examine the structure of the Organization itself and seek the causes of weaknesses which in practice have developed, it is no less important to consider the changed circumstances and conditions in which the United Nations as an organization operates to-day, compared with those existing when it was established. It is true that the repeated failure to reach agreement on the revision of the Charter has proved a great hindrance and disappointment—this is especially so since, more clearly than the Covenant of the League of Nations, the Charter provides expressly for its own revision and modification in the light of experience.

Some of the changed conditions, indeed, are to be found in the United Nations itself. While China remains outside, the fifty nations represented at Dumbarton Oaks have grown to 114. The increase does not represent any substantial new areas or populations, but the proliferation of former dependencies into independent States with their own representation. The change, moreover, has been largely within the past decade: equatorial middle Africa is now represented, for example, by 30 independent Black States, and of these only two were independent before 1956, and 19 have been created since 1960.

It is natural enough that a strong spirit of nationalism should run among the new nations, but nationalism goes ill with international statesmanship and co-operation. Further, the new nations are inexperienced as yet in world affairs: perhaps the most serious charge that could be levelled against the former Colonial Powers is that they did so little, and that late, to prepare their dependencies for independence, even when, as with Britain, the goal had long been accepted. This comparative political immaturity of the new nations constitutes a great danger, as it can tend to political irresponsibility. The resources required to support the United Nations and make its policies effective remain, by and large, with the Great Powers.

That position has to be recognized and it should be noted, in passing, that this extends also to scientific policy, as has been recently emphasized (*Nature*, 201, 327; 1964). The influence of science, however, is even more significant in two other factors which have transformed the world since the San Francisco Conference. The first is the appearance of the nuclear bomb, which was only used for the first time well over a month after that Conference. The decision to use the bomb introduced a new and unforeseen factor into the world situation, to which adequate thought was certainly not given by statesmen, although it would appear that some scientists did so. The second factor, and like unto the first, is the increasing pace of technological change of all kinds, of which a noteworthy instance is to be seen in the developments leading up to the *Early Bird* satellite transmission, which allowed Pope Paul's address to the General Assembly to be seen and heard, as he delivered it, thousands of miles away.

Development and change on this scale and at this rate must profoundly affect the relations between nations and the institutions through which those relations are organized. There has indeed been a remarkable growth in the activities of the various international scientific unions and the organizations through which those activities are conducted; so much so, that considerable expansion in the resources provided for the International Council of Scientific Unions is imperative if those activities are not to be circumscribed. The advent of nuclear warfare has intensified problems of disarmament; and its control, quite apart from all the psychological consequences and the problem of preventing the spread of nuclear weapons, is probably one of the most urgent and important problems of our times.

All this, however, has not made the United Nations redundant but more essential than ever if mankind is to survive. There is no other such viable international body reflecting the growing interdependence of the world and providing on an adequate scale the framework for the immense variety of inter-relations to-day. If it were dissolved it would be imperative to create immediately just such another institution as the alternative to world chaos. That this is realized is one hopeful feature in a gloomy situation.

Consider first, however, some of the consequences and implications of these scientific and technological developments of the past twenty years. The advent of nuclear weapons has increased the complexities of any steps towards disarmament, while at the same time it has undoubtedly had a restraining effect through the widespread realization that mankind cannot risk being involved in a nuclear war. If this problem could be solved the prospect of nuclear power has much to offer the world, though probably to a different extent in different parts of the world.

Here, however, we see that the real issue is the use which mankind makes of the new powers that have been placed at our disposal, whether they are used for good or for ill. The improvements in speed of travel and communication which, for example, enabled the Pope to reach New York so swiftly and allowed him to be seen and heard by millions when addressing the General Assembly, can be used to enable the Security Council to meet swiftly to deal with any emergency—if its members so desire. The techniques of television or broadcasting can be used thus for purposes of goodwill and to promote public understanding: they can equally be the sounding

board for propaganda, for misrepresentation and for fostering ill-will.

Finally, it should not be forgotten that the United Nations was founded deliberately as a democratic institution and such institutions make high demands on those who belong to them. They cannot function without discipline and fair-mindedness and a respect for the rights of others. The eruption into the General Assembly of a considerable number of those who, as yet, have had little experience of practising those qualities, of fostering the self-discipline, cannot but produce strains, misunderstanding, and failure to equate power and responsibility, and to forget that who wills the end must will the means. Time and experience are required to develop the qualities to use a democratic institution and to make it work effectively. If the demands made on the patience and sympathy of the old and more experienced democracies may sometimes seem heavy, if not excessive, it is at least encouraging that the new nations should be eager to use the United Nations as their main instrument and platform. That way there is at least hope.

If then the United Nations has ceased to be what its founders expected it to be, there is another side. In those twenty years of disappointment and frustration rather than failure have come the new opportunities provided by nuclear power, the exploration of outer space, the development of the backward parts of Africa and Asia, the acceleration, if not transformation, of communications; these opportunities which call imperatively for some sort of co-ordinating body have not been entirely missed by the United Nations. With some political failures there has been an immense advance in expert collaboration, within which to-day the International Council of Scientific Unions functions, and of which it is well aware. Some of this activity may be humdrum, but it is none the less vital, and the operations of the International Atomic Energy Agency, of the International Labour Organization, of the Food and Agriculture Organization, of the World Health Organization and of the United Nations Educational, Scientific and Cultural Organization are widely known. Some of them are indeed direct descendants of organs of the League of Nations and testify to the greater ease of international collaboration in technical as opposed to political matters. In fact, it is largely the influence of scientists that has led to what slender progress has yet been made in the control of nuclear weapons.

That is one important reason for welcoming the emphasis laid on technical assistance and the attention being given to the formulation of scientific policies for the developing nations. It is true that the spirit of the Lagos Conference on the organization of research and training in Africa in relation to the study, conservation and utilization of natural resources (*Nature*, **205**, 1141; 1965) was deeply disappointing as well as unrealistic. It gave little indication that Dr. S. Dedijer's wise counsel was receiving the attention it deserved. Nevertheless, the fact remains that the developing nations are using the United Nations as a means of obtaining help from the richer countries without becoming open to political pressures and also of securing important reforms in the system of world trade.

In this last-mentioned connexion, the solidarity of some 75 nations at the United Nations conference on trade and development should not be overlooked, nor indeed the skilful diplomacy of Britain which contributed so much to avoid a split and to help the United Nations to retain its chance of being a principal instrument for solving a large and explosive world problem. Since then, a most constructive report has come from the Advisory Committee on the Application of Science and Technology to Development. This body was established by the Economic and Social Council in January 1964 to follow up the work of the Geneva Conference in the previous year on the Application of Science and Technology for the Benefit of the Less Developed Countries (*Nature*, 207, 897; 1965). Here again are ample opportunities for constructive work, given goodwill and determined co-operation.

All this amounts to really impressive opportunities, and especially for Britain. Certainly, it should not be forgotten how much Britain has already contributed since the organization was founded. She is, in fact, the second largest contributor in cash and exceeds even the United States in the provision of experts who have served with the United Nations in the technical fields. This summer Britain gave a further lead with an unconditional gift of ten million dollars to help the United Nations out of its financial difficulties. However much more Britain might or should be doing, it is no service to peace to disparage or make party political capital of what she has hitherto done.

Where perhaps Britain—and others—have failed is in the tepid support given to the International Co-operation Year and the Development Decade. Half-way through that decade the target of 151 million dollars set in 1961 is still unachieved. Neither Britain nor the other members are really using the United Nations to do the jobs for which it was designed and where political obstacles are unsubstantial. That is why it is not enough for the emphasis to be placed on peace and peace-loving. We need not underrate the influence of Pope Paul's noble address, which may well extend far beyond his own Church or even Christendom. Where the way forward may seem at the moment to be blocked, we should be using to the full the opportunities for advance open elsewhere, and especially for bringing together in practical effort or consultation men and women of different nationalities.

Here the very rapidity of advance since the Charter was drafted at Dumbarton Oaks and then signed at San Francisco places a greater responsibility on the shoulders of the scientist and technologist. The opportunities which are theirs to-day are immensely greater, and though time may still be required before the effect of their co-operation reaches up to the highest levels, it can already begin to lay firm foundations for wider and deeper understanding. The Pugwash Conferences have at least indicated some of the possibilities. The challenge, moreover, is to professional institutions as well as to individual scientists and technologists. Their colleagues in the new nations will require the support and guidance which such institutions in the older countries can give if they are to organize themselves so as to influence effectively the Governments of their own countries and shape policy on lines that will promote the fullest development and use of indigenous resources. There are still large opportunities of personal service overseas which can contribute to international goodwill and understanding, and there is the responsibility that rests on all citizens to see that Britain's contribution to technical assistance and in other fields is generous.

If it is true that Britain should be leading the United Nations, part of that leadership must be contributed from the ranks of scientists and technologists. This is not to deny that realistic and constructive thought must

be given to the outstanding difficulties. Peace-keeping operations in the future will not always be agreed by everyone, and when some members have an objection to a particular operation, it is absurd and impractical to insist that they should be financially responsible for it. Moreover, while from time to time proposals will no doubt be advanced like those recently made for a world centre for medical research or an administrative staff college, which are of dubious merit on professional grounds, it should always be remembered that success in one place or field will lead to demands to use the United Nations and its agencies for others. All countries should be wary of squandering on dubious projects their resources and especially those of the United Nations, but where the effort is worth while, their response should be wholehearted, imaginative and sustained. That is the road to peace.

NATURALLY OCCURRING PHENOLIC COMPOUNDS

Biochemistry of Phenolic Compounds

Edited by J. B. Harborne. Pp. xi+618. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 126*s*.

BIOCHEMISTRY of Phenolic Compounds is a literary symposium which enjoys the advantages, and suffers the defects, of the type. It has been possible to gather together contributions from experts in their respective fields, hailing chiefly from the United States and the United Kingdom, with two Australian authors. But these are of unequal merit, ranging from a rather scrappy and ill-arranged selection of examples to well-ordered short monographs. For this reason the work is unlikely to be read *in toto* by students, though it must prove of the greatest value to research workers in any of the special fields discussed.

Few recent extensions of the organic chemistry and biochemistry of natural products have been more surprising than those recorded in relation to plant phenolics. Perhaps it was too readily assumed that these regions had been rather fully explored and that, in any event, their inhabitants were not particularly interesting. If so, both assumptions were quite incorrect. The variety and significance of phenolic substances is now seen to be great indeed.

This change of attitude is primarily due to the introduction of new techniques, particularly the chromatographic separations, which have injected a new precision into descriptive subjects such as those treated under anthoxanthins and anthocyanins. The results of surveys made with the use of these clearer glasses have given us not only a more accurate idea of composition, but have also provided taxonomic data and helped notably with certain genetic problems.

Chapters of fundamental importance to the worker in the chemistry of natural products are those of Margaret K. Seikel on the isolation and identification of phenolic compounds in biological materials, of J. B. Harborne and N. W. Simmonds on the natural distribution of phenolic aglycones, and of J. B. Harborne on the natural distribution of phenolic glycosides.

The first-mentioned covers the topic from *A* to *Z* and is an admirable account of it. The only useful tip, known to me, and not here mentioned, is the formation of good crystalline derivatives by combination of polyhydric phenols with *N*-methylstrychnine or *N*-methylbrucine.

The two chapters of which the editor is author and part-author are central to the theme, since without them the edifice could not have been constructed. The brilliant pioneering work of Bate-Smith at Cambridge must be recalled in this connexion. An example among the anthocyanins will serve to show the kind of progress made. Many of these pigments are well-characterized by their properties, including habit of crystallization. This is the case with chrysanthemin and pelargonin, to take two clear examples. But mecocyanin, isolated by Willstätter and Weil (1917) from flowers of *Papaver rhoeas* L., is by no means so well defined. Its discoverers showed that it hydrolyses with formation of cyanidin and two molecules of glucose. When chrysanthemin was synthesized, it became clear that mecocyanin must be a cyanidin 3-bioside, because its colour reactions were as near as possible identical with those of chrysanthemin (cyanidin 3-glucoside). Accordingly in 1934 the 3-cellobioside, maltoside, lactoside and gentiobioside were synthesized. These pigments closely resembled each other but showed slightly different distribution ratios between partially miscible solvents. The gentiobioside and mecocyanin came closest. But later the chromatography of carefully hydrolysed mecocyanin showed that the sugar was sophorose, that is 2-glucosidylglucose instead of the 6-derivative. Sophorose was first isolated in 1940.

The advances in chromatographic technique led at once to their application to genetic problems and, as R. E. Alston shows, the increase of visibility on the road, while clearing the way, has opened up fresh vistas. It cannot, however, be claimed that much progress has been made towards a real understanding of the basis of heredity in these fields. The facts have been clarified and arranged and this is an important preliminary to future generalization.

Useful summaries of what is known are also contained in two chapters on the metabolism of phenolics. This is, however, a part of general biochemistry and it seems somewhat artificial to concentrate on the phenolic group in all cases.

More specific to the subject-matter are the four highly interesting chapters on aspects of the biosynthesis of phenolic substances. The major pathways, recorded by A. C. Neish, are those starting from acetate (malonate), shikimic acid (or both) and the isopentane route. The part played by each is evaluated as the result of application of various experimental methods, of which the most important is the use of isotopic tracers. The general reader as well as the specialist will be fascinated by the tale that is unfolded. S. A. Brown describes the use of these and other methods to elucidate the biosynthesis of tannins and lignin. The work mentioned has not only solved the problem set but also has reflected light on the structure of these important substances themselves.

The final chapters on phenolics in relation to their pharmacology, physiology, pathology, and even taste are doubtless of high interest but look as if they had been added for the sake of completeness.

This compendium, with some faults and many virtues, is primarily of interest to chemists and biochemists and will be a standard work of reference for them for many years to come. Its production reaches the high standard which we have been led to expect from the publishers. There are few errors, the structural formulae being especially clear and accurate. ROBERT ROBINSON

HIGH-PRESSURE PHYSICS

Physics of High Pressures and the Condensed Phase

Edited by A. van Itterbeek. Pp. xv+598. (Amsterdam: North-Holland Publishing Company, 1965.) 140*s*.

THE growing interest in high-pressure studies has been reflected in recent years in a number of books, but there is no text dealing in such detail with the particular aspect chosen in this collection of articles by different

authors. The title of the book is somewhat misleading since most of the chapters deal with systems at low temperatures and moderately high pressures, and not all the topics are relevant to the condensed phase, for example, the articles on the spectra of gases. The standard is high, at the level of appeal to research workers. Since the chapters appear to be independent, with little cross-reference, they will be reviewed seriatim.

Chapter 1, by L. Deffet and L. Lialine, on "High Pressure Techniques in General", is limited to a rather perfunctory account of methods of closure and manometric devices, followed by a good summary of theory and observation on the elastic equilibrium and plastic flow of thick-walled cylinders. Ultra-high pressure techniques are not considered.

The mechanical properties of metals at low temperatures are treated by J. F. Watson, J. L. Christian and A. Hurlich in Chapter 2. This field has recently become important in relation to cryogenic work on missiles, hydrogen bubble chambers, etc. After a brief summary of theoretical principles the major part of this chapter consists of a detailed report on the behaviour of various alloys.

Chapter 3, by J. M. H. Levelt Sengers, consists of a critical appraisal of the experimental methods for the determination of the equation of state of gases at low temperatures and at pressures greater than a few atmospheres. An unusual feature is the report, about a page for each, on ten research centres throughout the world. A repeat is given of an account of manometric devices discussed in Chapter 1.

Chapter 4, by O. Verbeke and A. van Itterbeek, on "The Equation of State of Gases", is confined in the experimental section to work done at Leuven, Belgium (already reported on two pages in Chapter 3). This section is followed by a useful survey of data for the liquid vapour pressures of hydrogen, neon, oxygen, nitrogen, argon and methane and valuable compilations of thermodynamic data. In correlating these data more use could have been made of the principle of corresponding states. The final section comprises a summary of the various theories of the liquid state, including quantum liquids, and some comparison with observation (the authors state (p. 155) that the Monte Carlo method has not yet been applied to the liquid phase).

J. W. Stewart (Chapter 5) tackles the properties of "solidified gases" (that is, those substances which are gases under normal conditions) at high pressure and at temperatures less than 200° K, covering melting curves, compressibility, phase equilibria (surprisingly different for CH_4 and CD_4), etc. There is a useful summary of theories of melting.

After Chapter 4, with 23 pages on the theory of liquids, it is somewhat surprising to revert to this topic in Chapter 6 by A. Münster. However, the treatment is more sophisticated, although relaxation phenomena are omitted. Considerable attention is directed to the Monte Carlo method. As is usual in this rather unsatisfactory field, examples are limited to simple non-polar molecules.

Liquid and solid helium are rightly given separate chapters (respectively by R. H. Sherman, Chapter 8, and J. S. Dugdale, Chapter 9), since the quantum behaviour and choice of isotopes give a rich field of study. The bizarre properties of helium are excellently treated in these two chapters; the most compressible of solids, helium is often taken as a model substance from which the properties of other substances may be inferred in inaccessible regions. J. S. Dugdale rightly warns research workers against too slavish an adherence to this principle. Much remains to be done in this field; for example, why is an open body-centred cubic structure adopted near to 0° K?

"The Velocity of Sound in Dense Fluids" by W. van Dael and A. van Itterbeek (Chapter 7) and "Transport Phenomena" by O. van Paemal (Chapter 10) treat kinetic phenomena in fluids, often at low temperatures. The former chapter has the more detailed theoretical analysis.

Chapters 11 and 12, respectively by B. Oksengorn, H. Vu and B. Vodar on "Optical Aspects of High Pressure Investigations" and by J. P. Colpa on "Induced Absorption in the Infra-red", might have been combined; indeed the last-mentioned author devotes some space to the work of Vodar *et al.* already treated in Chapter 11. These chapters are concerned with molecular interactions in gases and their effect on spectral transitions, and such optical topics as the effect of pressure on the refractive index, rotatory dispersion, etc., are omitted.

The final two chapters on superconductivity (M. Levy and J. L. Olsen) and the electrical properties of metals and semiconductors (G. Landwehr) are, perhaps, somewhat out of place in this volume since the range of pressure is so much greater, extending to 600,000 atm. for measurements of conductivity, and only the former chapter is confined to low temperatures; nevertheless they are both useful summaries.

The treatment throughout is authoritative and the production excellent (although an author index would have been useful and the subject index is inadequate). In spite of the repetition, the occasional infelicities in the English, and the high price, this book can be recommended to all high-pressure workers, as it is packed with useful information, although much of the book is concerned with a rather limited range of molecules, the lighter inert gases and a few others of simple structure. The region of moderately high pressure, with which most of the book is concerned, provides a very satisfying study, since high precision is possible in the measurements and theory need not be unduly stretched. The editor is to be congratulated on bringing together this collection of articles which should stimulate further research—much remains to be done, for example, on the critical state.

R. S. Bradley

SPACE DYNAMICS

Dynamics of Rockets and Satellites

Edited by G. V. Groves. Pp. xi+313. (Amsterdam: North-Holland Publishing Company, 1965.) 80*s*.

THIS volume is a record of the lectures at the international summer school on the "Dynamics of Rockets and Satellites" held at Cambridge in July 1963. The book begins with a short section on the equations of motion of a rocket, by D. S. Carton. This is nicely balanced in the next chapter by an account of an actual design, the European Launcher Development Organization's launching vehicle, by H. G. R. Robinson. Chapters 3 and 4 return to mathematics, with J. M. J. Kooy discussing the dynamics of controlled rocket launching, and E. Stiefel dealing with many-body problems, as applied to interplanetary and circumlunar flight. In Chapter 5, R. H. Giese outlines the essentials of satellite tracking and orbit determination.

The next two chapters are concerned with orbital perturbations. M. J. Davies derives Lagrange's planetary equations and shows how they can be used for determining the effect of the atmosphere on orbits. W. M. Kaula propounds an elegant and generalized method for evaluating gravitational perturbations and also touches on the effects of radiation pressure, which have so far proved resistant to elegant formulation. The theme of the final two chapters is the rotational motion of a satellite about its centre of gravity. W. G. Hughes discusses attitude stabilization, including the torques acting, the methods of sensing attitude and control actuation. The subject of the last and longest chapter, by A. J. Sarnecki, is the dynamics of rigid-body rotation: a thorough and illuminating exposition of vectors and tensors is followed by a description of the rotational motion of typical satellites.

Most of the individual contributions are of a high standard and the complete book forms a valuable conspectus of space dynamics. In organizing the conference

Dr. Groves chose both subjects and lecturers judiciously; and in editing the book he has managed to avoid many of the perils of multiple authorship, though some overlapping among the contributions inevitably remains. The book would form a sound basis for an academic postgraduate course on space dynamics: it is, however, too advanced for an undergraduate course, and some of the contributions are of greater academic than practical interest. The book is well printed and the layout is pleasing.

D. G. KING-HELE

MATERIALS AT HIGH TEMPERATURES

Thermal Stress

Edited by Dr. P. P. Benham and Dr. Russell Hoyle. Pp. x+382. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 90*s*. net.

Plenum Press Handbooks of High Temperature Materials

No. 1. Materials Index

By Peter T. B. Shaffer. Pp. xx+740. 17.50 dollars.

No. 2. Properties Index

By G. V. Samsonov. Translated from the Russian. Pp. xii+418. 22.50 dollars.

No. 3. Thermal Radiative Properties

By W. D. Wood, H. W. Deem and C. F. Lucks. Pp. 470. 17.50 dollars. (New York: Plenum Press, 1964.)

THERMAL Stress is a symposium based on a conference held at the Imperial College of Science and Technology, London, in 1962, on the effects of temperature on the stresses within a body and on the properties of the component materials. It is divided into three parts, the first giving a résumé of the theoretical aspects of thermal stress, the second concerning thermal cycling and creep but also including a chapter on materials at high temperature, and the third detailing some industrial examples of thermal stress problems and their solution.

Like most published symposia, the book can be criticized for a lack of balance and completeness in dealing with its subject. The treatment of the high-temperature properties of materials, for example, is cursory, occupying only two out of twenty chapters, and would probably have been better omitted altogether. Regarded as a collection of papers, however, the book performs its function of presenting the thinking and results of those active in the field.

The *Plenum Press Handbooks of High Temperature Materials* provide, in three volumes, a wealth of data on those metals and ceramics that are used or usable at elevated temperatures. Volume 1 lists more than five hundred different ceramic materials such as carbides, borides, nitrides, sulphides, phosphides, silicides, and oxides, giving for each such structural, chemical, mechanical, thermal and physical data as could be culled from the sources consulted, these being mainly U.S. Government reports. The data available about a given substance vary enormously and the reader may be frequently disappointed in not finding the information for which he seeks. The book does not claim, however, to be exhaustive, and the author, Dr. P. T. B. Shaffer, is to be complimented on providing a good start to the classification of high-temperature materials and their properties.

Volume 2 in the series is the translation of a Russian work by Prof. G. V. Samsonov in which data on refractory materials are classified according to properties rather than materials. It has a brief but useful introduction followed by data on structure, composition, density and thermal stability. Chapter 2 deals with thermal properties of all kinds, and the succeeding chapters cover in turn the electrical, optical, mechanical, chemical and refractory characteristics of these materials. Oxides are not covered in this book as they have been dealt with elsewhere. A final set of tables gives examples of the application of refractory compounds. This work is more exhaustive than Volume 1 with more than 1,300 references compared with less than 500 in the latter, but the two volumes are clearly complementary and together provide an indispensable source of collected data.

Volume 3 deals with the thermal radiative properties of high-temperature materials and contains data on titanium and its alloys, stainless steel, iron, nickel and cobalt and their alloys, chromium, columbium, molybdenum, tantalum and tungsten with their alloys, coated materials of many kinds, ceramics and graphites. For each material the data provided include a graph of emittance (spectral or total) against temperature (given unfortunately in degrees Fahrenheit) together with information on the methods and conditions of measurement and, of course, the reference. In many cases graphs of normal spectral emittance against wave-length at some reference temperatures are also supplied. There are introductory chapters on "Fundamentals and Definitions" and on "Methods of Measurement", the latter being a brief survey backed up by thirty references to the literature.

E. H. ANDREWS

THE WORLD OF FISH

A Draught of Fishes

By Dr. F. D. Ommanney. Pp. 254+23 photographs. (London: Longmans, Green and Co., Ltd., 1965.) 36*s*. net.

SOME twenty-three thousand species of fish inhabit the waters of the world, more than those of all the other vertebrates put together. Their habitat covers more than 70 per cent of the surface of the globe, and ranges from the sunshine and surf of the shore to the black death-like calm of the abyss. The fishes—using the word in its widest vernacular sense—are the oldest class among the living vertebrates, and have had many million years more than the other classes to evolve in adaptation to their infinitely varied environment; though time has apparently stood still for many of them since the Carboniferous, natural selection must yet be acting on the combinations and permutations in the gene pool of others. This great host, not only of species but also of individuals, provides a subject well suited to such an accomplished writer as Dr. Ommanney, who has spent many years in research on improving the yield of commercial fisheries in many parts of the world.

The book opens with a chapter sketching in the oceanographic background of the fish's world and of the fisheries that exploit its wealth. The various forms of fishing gear and their methods of use are then described, from the latest European *V-D* trawl, and the ships equipped with radar and fish-finding echo-sounders that use it, to the simple traps and fish-weirs so commonly used on tropical coasts—not that simple "fixed engines" are extinct in Europe, for the wicker putchers still used in the Severn Estuary can be little different from the fish traps no doubt fished there when Julius Caesar first landed, or perhaps in neolithic times or even earlier. Dr. Ommanney intersperses his chapters on such subjects as shoaling, the colours of fishes, the abyss and the inshore waters, fresh waters, and the plankton feeding fishes, with others describing the different researches on specific fisheries, and the exploratory voyages undertaken in their prosecution, with which he has been associated. The last are among the best in the book, for his vivid style conveys the essential flavour of life in a trawler on the northern grounds off Iceland and Bear Island or with the tunny men of Brittany in the Bay of Biscay. The fisherman's world is

poles apart from the ordered routine of the landsman, who can have no inkling of its distance from his way of life; but, rough and uncomfortable as the life of a fisherman may be, the ceaseless struggle against the unending hostility of the elements and the ever-changing beauty, probably seldom consciously appreciated, of their infinite variety bring a primitive satisfaction that drives men to sea again and again in spite of all sea-born vows to swallow the anchor for good.

The last two chapters discuss the possibility of farming the sea, and the future of fisheries and man. The artificial cultivation of fresh-water fishes has a long history and a bright future, but no more than the first tentative experiments have yet been made in cultivating marine fishes—the artificial rearing of fry, the transplantation of young fish to rich feeding grounds, and the fertilization of semi-enclosed areas of the sea. One wonders whether pouring the untreated sewage of much of northern Europe into the North Sea can have had any effect in making it one of the finest fishing grounds of the world, or whether the suggested ploughing of the bottom to stimulate the production of the benthos which forms the diet of so many food fishes could increase the harvest. The human population explosion results in an ever-increasing demand for food protein; Dr. Ommanney speculates about the possibilities of satisfying the demand with the products of the sea, and on the effects of the radioactive wastes that we dump into the depths in 'leak proof' containers when inevitably they do leak. "There seems to be no limit to what man may achieve with his technology and his formidable know-how. . . . Nevertheless, the abuse of his own know-how threatens his very existence and already casts a lengthening shadow which reaches even to the uttermost depths of the sea."

Dr. Ommanney's book will be enjoyed equally by the professional biologist and by the layman for whom it is primarily intended. It is well illustrated with coloured and monochrome photographic plates and with numerous line drawings of excellent quality. There are, however, several howlers that a more careful final revision might have avoided; the Weberian ossicles have certainly not become the hearing bones of the ear of higher animals (p. 45), the claspers of elasmobranchs are not, as Agassiz mistakenly supposed, "pressed together so that their grooved inner faces form a tube along which the sperm flows" but are inserted singly into the female passage (p. 73); no carefully measured Basking shark has reached "a length of 40 feet", 30 ft. is nearer the mark (p. 79); the marine lancelet *Branchiostoma* is not the same creature as the "sand launce", which is the sand eel *Ammodytes* (p. 197), and Fig. 19 purporting to show gill rakers is a failure. These regrettable lapses form the few shadows that contrast with the brilliance of the rest of the book.

L. Harrison Matthews

PURPOSE AND MEANING OF A UNIVERSITY

The Modern University

By Prof. G. L. Brook. Pp. 192. (London: André Deutsch, 1965.) 25*s*. net.

PROF. G. L. BROOK, Smith professor of English language and modieval English literature in the University of Manchester and a Pro-Vice-Chancellor, has written an account of university problems to-day similar to that of 'Bruce Truscott' some twenty years ago. There is the same ease of expression but more authority as well as balance about the whole book. It is a little disappointing, therefore, that the production of such an admirable book should fall considerably short of what was possible in the midst of the War at a third of the price.

Prof. Brook's opening chapter gives a brief discussion of the purpose of a university, and this, together with the following chapter on entrance requirements, and an appendix on the organization of a modern university, should make plain to prospective students, and their parents, much that sometimes perplexes them. In the succeeding chapters he writes on students and on lectures and tutorials with a refreshing sanity and humour, as well as with balance; his remarks could be disconcerting to the extremist. In general, however, the most valuable chapters in the book are those which deal with books, with professors and lecturers and with academic freedom. The first will be welcomed by those urging more generous provision for university libraries. Librarians should be encouraged by the clear understanding shown of their difficulties and also of the problem of book-buying by students. The second corrects a misleading impression conveyed by 'Bruce Truscott' and should assist in giving a clearer understanding outside the university of the problems involved in the relations between research and teaching. Together with the final chapter on expansion, it brings down to earth some of the wilder statements and arguments of those who seek to determine university expansion by service to political doctrine rather than by reference to public needs and the real nature or purpose of a university.

For the chapter on academic freedom alone *The Modern University* would be welcome. Prof. Brook's exposition of the issues, including those aspects arising from the relations of university teachers with one another and the amount of freedom that students should enjoy, could scarcely be improved. He sees the danger at present as so real that our universities will do well to keep the very considerable freedom they have, and he believes that the chief threat comes from well-disposed persons, genuinely interested in education but who value other things more highly. The threat from outside is largely the result of the success of universities, and Prof. Brook shows clearly how the ear-marked grant can act in this way. The greatest single external threat to academic freedom to-day is the dependence on the State for so large a proportion of the university's income. The greatest internal threat is indifference among university teachers, or the practice of 'taking it for granted'; here he includes the fact that the scholar is reluctant to take his or her share in administration. Nor does he believe that the University Grants Committee will by itself suffice. For the defence of academic freedom it is necessary to convince public opinion that the State ought not to exercise the power it possesses. He adds that accordingly maintenance of a high standard of administrative efficiency under academic control is a most important safeguard of academic freedom, that many important decisions in university affairs should not rest entirely in the hands of one man, and that students, as well as teachers, have rights to be safeguarded.

Prof. Brook closes this chapter on a note of responsibility addressed particularly to the university teacher. That is the keynote of his final chapter, which includes many frank but uncomfortable words for student as well as teacher and administrator. He brings a timely pragmatic point of view to bear on the whole problem of expansion, arguing that the important question is whether potential students can profit by a university education sufficient to justify the considerable expenditure of time and money involved. He reminds us of the limits set to expansion by the availability of university teachers of the right calibre, and that the number of students who could profit from some other form of higher education is very much larger than the number of those whose proper place is in a university. He has constructive suggestions for breaking down existing barriers between school and university teaching. The whole tone of the book is a complete answer to those who accuse the university of being conservative, out of date and unwilling to face modern opportunities. Prof. Brook is to be congratulated on a most readable and timely book.

R. Brightman

MIMICRY AND THE EVOLUTION OF ANIMAL COMMUNICATION

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DURING the hundred years since Bates's discovery of mimicry in 1862, countless numbers of mere suggestions, experimental analyses and intense discussions about the evolution, the consequences and even the existence of mimicry have been compiled. This literature will not be reviewed here. Instead, I would like to suggest that the study of deceptive signals and a re-defined mimicry-concept offer a strict method for reconstructing the phylogeny of social signals and that we have to study mimicry carefully with respect to the evolution of animal communication. This makes it necessary to reconsider the concept of mimicry under the aspects of modern ethology.

As early as 1890, Poulton drew a distinction between defensive or protective mimicry, as illustrated by wasp-like beetles, moths, flies, etc., in which the mimic is preyed on by a predator, and aggressive mimicry, in which the mimicking species is itself the predator and preys on some animal which is not afraid of the mimicked species. This is the case, for example, in the mimicry between the wrasse, *Labroides dimidiatus*, and the blenny, *Aspidontus taeniatus*[1], which I have studied in greater detail[2], because *Aspidontus* mimics even behavioural characters of its model. *Labroides* removes ectoparasites from the bodies, fins and buccal and gill cavities of other fish. These fish often show special 'invitation postures' and the cleaner occasionally displays in a sort of dance in which the caudal fin is spread and the posterior part of the fish oscillates up and down. Size, form, coloration, and even the dancing of the cleaner are simulated by *Aspidontus*, who thus succeeds in eliciting the invitation posture from other fish. Being a fin-eater, however, he then attacks instead of cleaning them.

Another case classified as aggressive mimicry is that of the eggs and young of parasitic birds which mimic the signals serving as species recognition marks and/or eliciting brood care in the host species. The orchids of the genus *Ophrys*, the flowers of which mimic the social releasers of female Hymenoptera and elicit sexual behaviour in the males, fall into the same category; they offer no nectar but only the opportunity for pseudocopulation to the male insects, who thus transfer their pollen.

Essential for both types of mimicry is the deceived partner, one of the normal reactions of which is 'misused'. This may be a flight reaction or a blocking of feeding in protective mimicry, and quite a number of different reactions in so-called aggressive mimicry. Often a feeding reaction is exploited as by the angler fishes, but many other behaviour patterns may also be exploited, such as the need for being cleaned (by *Aspidontus*), the copulatory reaction (by *Ophrys*), or the care of the young (by the various brood parasites). These examples show (1) that in spite of some existing mimicry-definitions no predator need be involved in a mimicry system, and (2) that the terms 'defensive', 'protective', and 'aggressive' are inadequate to cover the diversity of signals and reactions misused in mimicry. Moreover, though colours and forms of organs and movements as optical markers are most conspicuous to us, scents and sounds may be mimicked also, as Kullenberg[3] has shown in some orchids which even imitate the sexual odours of hymenopteran females, and as Nicolai[4] has shown in the whydahs that imitate the calls and songs of their host species. Therefore, instead of referring to pseudosematic or deceptive colours we should rather speak of deceptive stimuli or signals. Since the deceived partner may be responding as predator, or mate, or parent, or cleaning customer, it will be called simply the reacting signal-receiver or the receiver.

Mimicry, then, can be defined in the following way:

(1) A signal is emitted by two different signal-senders (S_1, S_2) which have at least one signal-receiver (R) in common that reacts similarly to both of them.

(2) One of the senders is called a model, the other a mimic (and the whole model–mimic–receiver system a case of mimicry), if it is profitable (+) for the receiver to give the reaction to one of the senders, but unprofitable (−) to react in the same way towards the other. That means that if the signals from the two senders could be distinguished by the receiver, individual experience and/or selection would favour different reactions. This is shown, for example, by the cleaner mimicry: *Aspidontus* deceives mainly younger fish, at least some of which then learn to distinguish between model and mimic and to avoid the latter. Constant learning by the signal–receiver results in a strong selection pressure against detectable differences between model and mimic.

(3) It suffices to define model and mimic as follows: the sender the signal of which elicits a reaction which has negative selective consequences for the receiver (which would be selected against if given to this sender only), that is the sender by which the receiver is deceived, is called the mimic, the other the model. These relationships may be symbolized thus:

$$S_1 \quad +R- \quad S_2$$

The selective consequences for the receiver of responding to the model are always positive (the reaction would disappear if it were at all unfavourable to the receiver). The mimic—if a living creature—always has a selective advantage (+) in releasing the reaction from the receiver (an unfavourable signal will disappear, too). If S_2 is the mimic, our formula automatically turns into:

$$S_1 \quad +R- \quad +S_2$$

(It is, of course, a matter of convenience whether one chooses the reaction or the signal as a reference unit; one could equally as well have considered the selective consequences for the receiver in receiving the signal from both senders and for them in emitting it.)

The selective consequence for the model eliciting and obtaining the reaction from the receiver may: (*a*) be absent, if the model is an inanimate object; (*b*) be negative, if the model is a worm, for example, usually eaten by the receiver and mimicked by another predator in order to catch the receiver; (*c*) be positive as in the wasp (who remains alive if it is avoided by the predator), or in the

cleaner (who feeds on parasites harmful to other fish), or in those hymenopteran females, the male-attracting signals of which are mimicked by certain orchids.

The simple definition given here overcomes the difficulties arising from some recent characterizations of model and mimic. It is irrelevant which of the two was first present. Poulton postulated that model and mimic should be sympatric. They need not be sympatric, however, but must only have a signal–receiver in common: a model might be in Africa and its mimic in Europe (or vice versa), functionally connected by a migratory bird. Another postulate was that mimics must be less numerous than their models. This means, correctly stated, that the receiver has to meet the mimic less often than the model, and is based on the assumption that one experience with the model has the same after-effect, the same 'weight' as has one with the mimic. This need not be so, however; in fact, the negative experience seems usually to be the stronger one. This negative experience may result from an encounter with the model (a wasp) or with the mimic (for example, the *Aspidontus*). In the latter case one would suppose that the mimic in fact has to be less numerous than the model[2], but there might very well be more wasp mimics than wasps.

Assuming that a predator learns to reject a conspicuously coloured protected animal from a single experience and—as in imprinting—without forgetting it, then the model-species has to sacrifice one individual per predator regardless of how many mimics there may be. Of course, the protective power of the model, that is the advantage of being its mimic, is reduced with increasing numbers of mimics, because the predator will eat larger numbers of them before his first encounter with the model. Furthermore, if the signal receiver reacts towards the signal via an 'innate releasing mechanism' (IRM) which cannot be modified by experience, each individual of the receiver species will be fully deceived by the mimic even if it never meets a model (this seems to be the case in some of the female-mimicking orchids). Thus, whether or not the reaction is eliminated in phylogeny depends on its usefulness to the species in other contexts.

In all cases the selective value for the mimetic characters increases, among other things, with the number of successful deceptions from one deception per receiver on. The orchids (and other mimicking flowers) are the only mimics known to me, however, that need at least two deceptions per receiver—in order to undergo cross-pollination. The disadvantage for the deceived species should, therefore, be minimized, as it is by the orchid-flower allowing only attempts at copulation and thereby parasitizing the strong reward offered by true copulations with a female. So, finally, the type of reaction given by the receiver has also to be taken into consideration.

Signal Standardization and Müllerian Mimicry

It will have become clear already that even fundamental characteristics of mimicry are determined mainly by behavioural properties of the signal–receiver and that, therefore, a precise knowledge of the identity of the receiver and a thorough ethological study of its behaviour are indispensable for the understanding of mimicry. Additional arguments for this will come from the following considerations which will show, moreover, that mimicry is not sharply distinct from, but gradually merges into, other sender–receiver communication systems.

First, since palatability is a matter of degree, there might possibly exist a whole range of increasing distastefulness even in the mimics, model and mimic in the extreme case being equally unpalatable and sharing the same warning coloration. This stage is called Müllerian mimicry which in our notation would be:

$$S_1+ \quad +R+ \quad +S_2$$

This is no true mimicry, however, since nobody is deceived and it is impossible to differentiate between model and mimic. But there is still another difficulty: all individuals of a given wasp-species look alike and all are equally protected. Yet this is not usually called Müllerian mimicry, simply because the signals were not independently, convergently evolved. Since, however, the male wasps have no protective properties[5] but retain their group-specific warning coloration, this clearly is Batesian mimicry, although model and mimic are conspecifics and their signals are homologous. Convergence of the signal-characters, therefore, is essential only for the so-called Müllerian mimicry, and thus Müllerian mimicry is distinguished from other cases of signal standardization.

Summing up, the typical (Batesian) mimicry merges into Müllerian mimicry if the difference between the selective consequences for the receiver of reacting similarly to S_1 and S_2 diminishes; and by homology of the signal characters it further merges into general signal standardization and such problems as that of typical intensity[6].

Secondly, insects may be protectively coloured by resembling a wasp or a twig, the first case being called mimicry, the latter mimesis. The difference again lies within the signal–receiver. If the mimetic signal does not release any reaction in the receiver, it falls under mimesis. This is illustrated by De Ruiter's neat experiments with stick caterpillars which by virtue of their close resemblance to twigs are protected against insect-eating birds[7]. As soon as there are too many caterpillars, however, the bird develops an interest in them and then also attacks real twigs. In our notation this would be:

$$S_1- \quad +R- \quad -S_2$$

S_1 being the caterpillar, S_2 the twig and (as shown by R's minus) the mimic. If then one positive experience of R with the caterpillar had the same weight as a negative one with a twig (and the signal remains unchanged) it will depend on the relative abundance of caterpillars and twigs whether all twigs are mistakenly exterminated or R's feeding reaction towards twig-like objects disappears, changing our formula into:

$$S_1+ \quad -R+ \quad +S_2$$

so that now the caterpillar (S_1) becomes the mimic.

This again illustrates the importance of R's reaction to decide correctly which is the model. This example further shows how easily an object (here the twig) may quite involuntarily become a mimic. This is further well illustrated by the common 'farming' relationship between ants and aphids. The siphones and cauda of the aphid correspond respectively to the bases of the antennae and labium of the ant, and the aphid's abdomen is mistaken by the ants for the head of a fellow ant, thereby eliciting the food-begging response which is identical with milking. Saturated ants in their turn even try to feed the abdomens of the aphids[8]. Aphid-species with reduced abdominal siphones use their hind legs as antennae-dummies, the movements elicited being originally defensive movements. This is exactly the way in which mimicry arises. Mimetic characters need not have evolved under the selection pressure of mimicking; in fact, their earliest evolutionary stages could not even have been brought about in this way. All the cases that I have been able to study so far could be traced back to an incipient stage of deceptive resemblance borne as a pre-adaptive atelic by-product of pre-existing species-specific characters, thus providing a point of attack for selection pressure. The 'dancing' of *Aspidontus* mentioned already originated as an up-and-down movement of the head which occurs in all blennies during a conflict between the tendencies to approach an object and to flee from it. This may occur in stationary fish as nodding or be superimposed on swimming, becoming a sort of dance. These movements usually have a special social meaning in threat or court-

ship, but in *Aspidontus* they are socially meaningless and so could become specialized as an interspecific signal[2]. That the characters of the mimicking signal secondarily evolve further under natural selection is usually admitted, though sometimes doubted, as is even the mere existence of positive selective consequences resulting from the deception of a communication partner. The simple reason for these doubts is that in order to analyse and understand a given mimicry-system one needs a rather comprehensive knowledge of morphology, behaviour, ecology and their mutual relationships in animals usually belonging taxonomically to different classes of the animal kingdom (for example, wasps (Hymenoptera)—flies (Diptera)—insect-eating amphibians, reptiles, birds and small mammals). To reconstruct the evolution of such a complicated communication system, one also needs a detailed acquaintance with a larger group of forms related to each of the animals involved. But, in fact, we are far from having all these data.

Intra-specific Mimicry

That positive selective consequences do result from the deception of a communication partner is, however, shown automatically by deceptive signals that have evolved intraspecifically. In the small characoid fish *Corynopoma riisei* the operculum of the male is elongated into a thin whitish stalk terminating in a small blackish plate. This is raised during courtship together with the operculum, and by its jerky movement resembles an edible object. This the female tries to grasp, and so comes into close contact with the male. Here mimic and receiver belong to one species. There are other cases (for example, the mimicking orchids or the parasitic whydahs) in which model and receiver belong to the same species. However, the safest conclusions can be drawn from intraspecific mimicry, in which model, mimic and receiver all belong to one and the same species. The best-known such example is the use of egg-dummies by the males of mouth-brooding *Haplochromis* species[9], a group of African cichlid-fish. These dummies are conspicuous yellow or orange spots near the base of the anal fin which precisely resemble the eggs of the particular species. They are displayed to the female during courtship and during fertilizing and elicit her reaction of snapping up eggs. During courtship they make the male more attractive to the female. In spawning they assure fertilization of the eggs, which are taken into the female's mouth immediately after they have been laid and before the male is able to fertilize them: in snapping at the 'egg spots' with her lips, the female inhales the sperm, thus fertilizing the eggs within her mouth.

Here the specific female behaviour pattern of gathering eggs into the mouth is elicited alternately by model and mimic several times in succession with intervals of only about 1–2 sec, and within the same spawning pit. This offers ideal opportunities for comparison, since environmental factors as well as the internal states of the animals remain roughly constant. Moreover, the undeceived and the deceived reactions together form a functional unit, the deception clearly having positive selective consequences for the species. Nevertheless, the receiver's minus towards the mimic in our formula remains correct, since the individual clearly is deceived.

Another example of intraspecific mimicry is offered by some Old World monkeys, especially the Hamadryas baboon, the females of which during oestrus display conspicuous swellings of the ano-genital region, which is presented to the males as an invitation to copulate. Presenting, however, serves still another social function, since it is used by both sexes as an important appeasement or 'greeting' ceremony towards higher-ranking group members. The males, in order that their presenting be as effective as that of the females, imitate the females' swelling by a conspicuously red coloration of their hind-quarters as a dummy oestrous-marking[10,11].

The Theoretical Importance of Deceptive Signals

To reconstruct the evolution of a case of intraspecific mimicry as a special communication system is relatively simple for one needs detailed knowledge of but one rather narrow taxonomic unit. This is important, because mimicry is an indispensable step in studying the evolution of animal communication. This study usually starts from conspicuously elaborated signals, which postulate a signal–receiver interested in them. The receiver will practically always have undergone a special moulding towards optimal receiving of the signal. One must, however, follow up the mutual adaptations of the sender and the receiver separately. This is easily done so far as the evolution of a reaction or a receiving mechanism is concerned in all predators that try to find their prey, and in all prey-animals that try to escape an approaching predator. The 'suppression' of signals may be studied in predators that try to sneak up on a prey unnoticed. However, the 'elaboration' of a signal (in which the receiver must, of course, be interested) can only be studied without co-adaptive interference from the receiver where the sender has a one-sided interest in the signal, which then can only be an imitation of either a signal developed in their common interest by the receiver and another signal-sender or a signal emitted by another signal–sender and only made use of by the receiver in his own interest. Both cases, by the definition given, are called mimicry. An additional advantage is that we know the model as the final stage towards which the mimic will evolve (so far as the signal characters are concerned), thus indicating the trend in evolution that is still operating and that probably will further elaborate the mimetic signals.

Finally, we may briefly consider social signals in general. It is legitimate to treat them as structures, even if they are motor-patterns[12]. Evolutionary 'new' structures are merely modifications of preceding structures; "the emergence of a new structure is normally due to the acquisition of a new function by an existing structure"[13]. In the same way the egg-signal in *Haplochromis*, which originally belonged to the context of brood-care, has taken over the new functions first of bringing the gametes together and, secondly, even of bringing the parents together; and it has given rise to a new structure, the egg-dummies. If all signals have emerged during evolution at one time or another as 'novelties' one may question for what they might have been mistaken *in statu nascendi* by their respective signal–receivers. To treat signals generally as having passed through an original mimetic stage renders studies of mimicry valuable tools for students of the phylogeny of signals and of animal communication. In this way I have found that another cichlid fish, *Tilapia macrochir*, deposits spermatophores: I considered the male's conspicuous genital tassel as an imitation of something which finally turned out to be the spermatophore[14]. It should be added, however, that it is not as yet quite clear whether the spermatophore truly is the model, because both tassel and spermatophore may be mimics of a still unknown model. Finally, there is the possibility of chain-mimicry, that is, of secondarily mimicking a mimic; however, research in this field is still in a very early phase.

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THE JUVENILE HORMONE*

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IN choosing this topic for the opening address to the third conference of European Comparative Endocrinologists, I had in mind the consideration of the juvenile hormone in general terms—as an agent with properties that must be of interest to all endocrinologists. I shall not therefore attempt a complete or specialized survey of this very complex subject, but will select only a limited number of points for discussion.

A Morphogenetic Hormone

The juvenile hormone is a morphogenetic hormone—one of the most outstanding morphogenetic hormones known. The existence of a hormone responsible for maintaining larval characters was first revealed by the fact that decapitation in young *Rhodnius* larvae causes premature metamorphosis[1]. Parabiosis showed that this hormone was freely circulating in the blood; and by removing progressively more and more of the anterior parts of the head from the insects used in the parabiosis experiments, it was shown that the hormone came not from the brain but from the corpus allatum—an endocrine organ that buds off from the ectoderm at the base of the mouthparts and comes to lie just behind the brain[1]. This conclusion was confirmed by the implantation of corpora allata from young (3rd- or 4th-stage) larvae into 5th-stage larvae. These 5th-stage larvae then moulted not into adults but into giant 6th-stage larvae[2].

The juvenile hormone acts directly on the epidermal cells responsible for laying down the cuticle at moulting: restricted local application of the hormone results in a restricted local patch of larval cuticle in an otherwise adult insect[2]. It is possible, for example, to produce adult insects with one larval wing[3]. The self-same cell has the capacity for laying down larval cuticle or adult cuticle. That is most clearly shown in the sensory bristles or hairs: the trichogen cells which form the bristles persist from one stage to the next; in the presence of a large amount of juvenile hormone these cells lay down larval-type bristles; in the absence of the hormone they lay down adult-type bristles, and with intermediate amounts of juvenile hormone, bristles of intermediate type are developed[1]. Under natural conditions intermediate forms do not commonly occur. There is a strong tendency for the insect to develop either larval or adult characters.

Experiments by many authors have shown that the juvenile hormone controls metamorphosis in insects of all kinds. In holometabolous insects, which have a pupal stage between the larva and the adult, the juvenile hormone again controls the morphogenetic change: a large amount of the hormone ensures retention or re-development of larval characters; absence of the hormone results in metamorphosis to the adult; the presence of a very small amount of juvenile hormone leads to the appearance of the pupal form[4]. This result not only confirms the importance of the juvenile hormone in controlling morphogenesis, but it illustrates in a most striking way a point often made by C. M. Child: that the same inductor substance can evoke totally different results depending on its concentration or the timing of its action.

Here again intermediate forms rarely occur in Nature. But they can be induced experimentally: if the corpus allatum is removed from the last-stage larva of the honey-bee[5] or of the giant silkmoth *Hyalophora*[6] the supply of juvenile hormone falls below the level necessary to produce the pupa, and monstrous forms intermediate between pupa and adult develop. Some caterpillars pass through a regular succession of morphological stages in successive instars. These forms also seem to be regulated by levels of juvenile hormone secretion—but the detailed evidence for this has not yet been fully worked out[7].

The Nature of Hormone-controlled Metamorphosis

It has long been recognized that the effect of the juvenile hormone is to control the realization or suppression of inborn potencies. In other words, it brings about 'gene switching'. In this regard it resembles the inductor substances which control morphogenesis in different parts of the body during differentiation; and the factors which lead to the differences in form of different individuals in environmentally induced polymorphism[8]. Indeed, there are a number of polymorphic changes in which there is evidence that the level of juvenile hormone activity may itself be involved, notably (i) the change over from the 'solitary' to the 'gregarious' form in locusts[9]; (ii) the switch from apterous to alate forms among parthenogenetic aphids[10]; (iii) the production of the soldier caste in termites[11,12].

Just how and where the juvenile hormone is acting is not known. Twelve years ago I wrote "it is a matter for discussion whether the simultaneous inheritance of the dual potentialities for larval and adult differentiation within these societies of cells is by way of the nucleus or cytoplasm or both"[13]. Eight years ago I submitted that the juvenile hormone "controls the manifestation of alternative genetically controlled forms" and I suggested that "it is possible to conceive it as being concerned in the regulation of permeability relations within the cells—in such a way that the gene-controlled enzyme system responsible for larval characters is brought increasingly into action when the juvenile hormone is present"[14].

The position is unchanged to-day—except that tissue-specific puffing patterns in the chromosomes of *Drosophila*[15], on one hand, and the theories of enzyme induction in bacteria as developed by Jacob and Monod[16] *et al.*, on the other, render us much more prepared to accept the idea of a primary action of the hormone at the level of the gene. Experimental evidence has yet to come.

Reversal of Metamorphosis

The question was early raised whether the genetic system responsible for the production of larval characters was still capable of re-activation by the juvenile hormone in the adult insect. In other words, whether metamorphosis can be reversed. Of course, this can only be tested by making the adult moult again by exposing it artificially to the moulting hormone.

In general it can be said that in most adult tissues it is not possible to induce any reversal of metamorphosis. But there are certain undoubted examples of such reversal: in the abdominal cuticle of *Rhodnius*[17] and of *Oncopeltus*[18], in the thoracic cuticle of the earwig *Anisolabis*, where the 'ecdysial line' characteristic of the larva can be re-induced in a moulting adult[19], and in the integument of Lepidoptera where larval cuticle can be re-induced in fragments of pupal and imaginal integument[20,21].

Reversal of metamorphosis is an abnormal phenomenon. There has been no selection for its occurrence and it is

* Substance of an opening address to the third conference of European Comparative Endocrinologists held in Copenhagen during August 1965.

not surprising that it occurs with difficulty. Likewise the production of stages intermediate between the normal stages in metamorphosis is an abnormal phenomenon and selection will have acted against it.

Time of Action of the Juvenile Hormone

The most effective moment for exposing the tissues to the juvenile hormone seems to be just before they begin the synthetic activities characteristic of the larval stage. Hormone administered too early is less effective—presumably because it is broken down in metabolism before the time for its action has arrived[22,23].

But there are certain effects which are induced in the cells long before they become manifest. A very small dose of juvenile hormone administered soon after feeding in the 5th-stage larva of *Rhodnius* has no effect on the type of cuticle laid down: normal adult cuticle is produced over the abdomen. But it does have the effect of ensuring the survival of the trichogen cells (almost all of which normally break down and disappear from the dorsum of the abdomen during metamorphosis), so that an excessive number of hairs appears on the abdomen of the adult[23].

The juvenile hormone has a comparable effect on the survival of the thoracic gland. The thoracic gland (the source of the moulting hormone) normally undergoes autolysis in *Rhodnius* within 24 h after moulting to the adult. This is a response to some hormonal factor in the newly moulted adult. But if the gland has been exposed to juvenile hormone during the pre-moulting period, it does not respond in this way and fails to undergo autolysis[24]. (The breakdown of certain muscles after metamorphosis in the silkmoth *Hyalophora cecropia* has recently been shown to be regulated in much the same way[25].)

Influence of the Juvenile Hormone on Behaviour

In certain caterpillars, behaviour is different in the final stage before pupation than it is before moulting in the earlier larval stages. The wax moth *Galleria* spins a tough cocoon before pupation, a flimsy web before a larval moult[26]. The sphingid *Mimas tiliae* crawls down the tree-trunk to the soil before pupation, but rests on the foliage before each larval moult[27]. These differences result from the presence or absence of juvenile hormone and are attributed to a direct effect on the nervous system. But the possibility remains that they could result from a nervous feed-back effect from other organs, for example from the distended silk glands.

Gonadotrophic Effects of the Juvenile Hormone

Secretion of the juvenile hormone ceases in *Rhodnius* in the 5th-stage larva before metamorphosis, but begins again in the adult. The hormone is then necessary for yolk formation in the female and for the full activity of the accessory glands in the male, which serve to produce the spermatophores to enclose the sperm[2]. In *Hyalophora cecropia*, in which the eggs are developed during the pupal stage, very little juvenile hormone is secreted in the adult female, but large amounts are produced in the adult male[28], which secretes a succession of spermatophores.

The precise nature of the gonadotrophic effect of the juvenile hormone is uncertain. The position is complicated by the fact that in some insects the secretion from the neuro-secretory cells in the brain seems to be more important than the juvenile hormone in ensuring yolk production. In the case of *Rhodnius*, G. C. Coles[29] concluded that the juvenile hormone acts on the fat body cells and serves to activate those components of the gene system which lead to the synthesis of the specific proteins that are discharged into the blood and are taken up by the oocytes and added to the yolk. In the male locust, *Schistocerca*, T. R. Odhiambo[30] considers that the juvenile hormone activates the many systems concerned in protein synthesis in the nuclei of the accessory glands.

Metabolic Action of the Juvenile Hormone

The juvenile hormone is often said to be a 'metabolic hormone'—by which is meant that it sets going metabolic processes, either of synthesis or of combustion, whether or not these are required for the working body. I am sceptical about the existence of metabolic hormones in this sense; I think they are unphysiological. I fancy that in most cases of this kind the hormone is setting in motion some physiological process, and the observed changes in metabolism are feed-back effects. (But, of course, abnormally large doses of hormones may have an abnormal pharmacological effect on metabolism which has little relation to their normal influence in the body.)

A well-known example of a metabolic effect of the juvenile hormone is the accumulation of reserves of fat and glycogen in the fat body of the grasshopper *Melanoplus* when the corpora allata are removed[31]. But Odhiambo[32] has shown that in the locust *Schistocerca* the juvenile hormone acts on the central nervous system and causes continuous activity. After allatectomy the insect continues to feed normally but it becomes inactive; consequently reserves pile up. The accumulation of reserves is not a feed-back effect from the gonads; the same effects are seen in males with or without their accessory glands.

In the blowfly *Calliphora*[33] and in the cockroach *Leucophaea*[34] the corpora allata are necessary to maintain the normal high level of oxygen consumption. But here again the juvenile hormone may be initiating some physiological activity (perhaps the synthesis of ovarial proteins) which demands increased oxygen consumption. In these cases it is not known whether nervous or muscular activity is changed. In the bug *Pyrrhocoris* the effect of the corpus allatum on metabolism is seen only if the gonads are present: it appears to be a feed-back effect from the activated ovaries which are demanding nutrients[35]. Likewise in *Rhodnius*, the accelerated rate of digestion in the presence of the corpus allatum is a feed-back effect from the developing ovaries[36].

In *Leptinotarsa* the adult female goes into diapause when the juvenile hormone is absent or the corpora allata are removed. The rate of metabolism falls to a very low level and egg development ceases. In this state the thoracic muscles and their mitochondria degenerate almost completely. When juvenile hormone is supplied everything is restored: the beetles become active, the ovaries develop, muscles and sarcosomes are fully regenerated[37]. The metabolic effects are profound—but just where the juvenile hormone is acting is not known.

It is, of course, self-evident that hormones can influence the body only by bringing about chemical changes in the cells. In this sense they are always 'metabolic hormones'. Within a few hours the moulting hormone restores nucleoprotein synthesis in dormant epidermal cells of *Rhodnius*[14]; the juvenile hormone seems specifically to induce the synthesis of yolk proteins in the fat body of *Rhodnius*[29]; as Gilbert[38] has recently shown, the juvenile hormone will induce the fat body of the cockroach *Leucophaea* to synthesize ovarian lipids. But these, and many similar effects, are elements in a pattern of development evoked by the hormone. They are not simply quantitative changes in metabolism unrelated to growth requirements—as is commonly implied by the expression 'metabolic hormone'.

Chemical Nature of the Juvenile Hormone

The large accumulation of juvenile hormone in the adult moth of *Hyalophora cecropia* provided a source of active extract. Ether extracts from the abdomen of the male moth gave an orange-coloured oil rich in juvenile hormone activity[28]. This material was utilized to develop methods of assay[3,22] and methods for concentrating the active principle by countercurrent separation[22]. These procedures were applied to extracts from many sources

and have shown that material with roughly the same partition properties, and with juvenile hormone activity, is widely spread throughout the animal and plant kingdoms: in the tissues of invertebrates and vertebrates, in higher plants, some bacteria and yeasts.

The material extracted from two of these sources, namely from the excrement of the mealworm, *Tenebrio*, and from yeast, was examined chemically by Schmialek[39]; the active principle was isolated and shown to be a mixture of *trans-trans*-farnesol and its aldehyde farnesal. The question arises whether the natural juvenile hormone in the insect has any relation to farnesol or is of a totally different nature.

At the present time the answer to this question is not known. Farnesol will certainly reproduce all the morphogenetic and gonadotrophic effects of the juvenile hormone in *Rhodnius*[40]. It is particularly effective if its stability is increased by blocking the alcohol end of the molecule in the form of an ether (for example, farnesyl methyl ether) or as the farnesyl acetone[41].

In the form of the methyl ether, a 0·06 per cent solution of the active *trans-trans* isomer is far more effective than the natural extract from *cecropia*. 1·2 μg will cause a partial retention of larval characters in *Rhodnius*—a dose of 5·2 μg/g of body-weight[23]. In *Antheraea*, 20,000 μg of the natural extract from *cecropia* will cause a partial retention of pupal characters—a dose of 4,000 μg/g of body-weight[42].

By repeated partition with methyl alcohol, chromatographic separation on silicic acid columns, followed by the crystallization of impurities, a 'non-crystalline fraction' has been isolated which will produce this same effect in *Antheraea* at a dose of 5 μg, that is, 1 μg/g of body-weight[42]. Accepting these results at their face value this product is about five times as effective as farnesyl methyl ether in *Rhodnius*.

Further attempted purification by gas-liquid chromatography leads to heat destruction of the natural substance, but one fraction isolated in this way had an activity twelve times that of the 'non-crystalline fraction'[42]. Meyer, Schneiderman and Gilbert[43] report similar results, showing pyrolytic breakdown of the material in the gas-liquid chromatography column, but with some highly active fractions. Röller, Bjerke and McShan[44] have isolated a well-defined active substance which is certainly not farnesol.

The significance of these results is uncertain. Assay methods for material that is exerting its action over a period of many days of development, and which is continuously being broken down in metabolism, have no very precise meaning. (By modifying slightly the means of administering the material I have recently repeated the results on *Rhodnius* as described here with half the earlier dose, that is, with about 2·5 μg of the active isomer of farnesyl methyl ether per gram of body-weight.) At the present time farnesol derivatives are the only compounds of known chemical composition with juvenile hormone activity. They were identified as the substance with juvenile hormone activity in the extracts of non-insect material. They have been found in the extracts of *cecropia* and other silkmoths. Until other known compounds have been isolated, I am inclined to hold to the provisional hypothesis that the active group in the juvenile hormone is indeed the triple isoprene unit of farnesol, and that this exists in the natural hormone in some form that has not yet been defined.

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OBITUARIES

Dr. H. R. Marston, F.R.S.

Dr. Hedley Ralph Marston died in Adelaide on August 25, the day before his sixty-fifth birthday, and the day on which he was to retire from his post of chief of the Division of Biochemistry and General Nutrition of the Australian Commonwealth Scientific and Industrial Research Organization. He was one of three brothers born in Bordertown, South Australia; his father was a Civil Servant. The family moved to Adelaide where he attended Unley High School. A school-fellow, Mark (now Sir Mark) Oliphant, attained high scientific distinction and remained a life-long friend. In 1934 Marston married Kathleen Nelly Spooner, who survives him. They had no children.

There were two rather unusual features about Marston's career. From first to last he held appointments in Adelaide; and his first degree was conferred on him *honoris causa*, some thirty-three years after his first academic appointment. When an undergraduate student of chemistry in the University of Adelaide, he chanced to meet, during a country walk, a stranger with whom he fell into conversation. This proved to be Brailsford Robertson, newly arrived to take up the chair of physiology and biochemistry in the University. Within a few days, Marston had joined Robertson's department as demonstrator, and during the next seven years assisted him in organizing the teaching of biochemistry and physiology in the University. In 1926, Marston sat for the B.Sc. examination in biochemistry and physiology and headed the honours list; but he was

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by then so fully engaged in research and teaching that he was unwilling to retrace his steps in order to comply with the regulations necessary for taking a degree. This rather unorthodox entry to the academic world in some degree recalls that of Gowland Hopkins, who was first an articled pupil to a public analyst: he moved to Guy's Hospital Medical School, where he took a medical degree, but only in time snatched from research and teaching commitments. It may be that academic life will be the poorer now that insistence on 'paper qualifications' has made such unusual débuts all but impossible. During these early years, Marston received much help from Archibald Watson, the professor of anatomy, of whom he always spoke with affection, as well as from Brailsford Robertson.

His first paper adumbrated the importance of the mitochondria as centres of enzyme activities—a concept since amply confirmed. In other papers he showed for the first time that the mammary gland of the monotreme echidna did indeed secrete true milk, described the first micro-method for the determination of guanidine and methyl guanidine, and published work on the sterol isolated from the 'giant toad stool'.

In 1927 he joined the Division of Animal Nutrition of the (then) C.S.I.R., newly formed under Brailsford Robertson. He was in charge of the Division between Robertson's death in 1929, and the appointment of Sir Charles Martin as chief in 1930. A close bond grew up with Martin, and when he returned to England in 1932, Marston was appointed chief nutrition officer, and placed in charge of the Nutrition Laboratory, which became part of the Division of Animal Health until 1944, when the Division of Biochemistry and General Nutrition was established with Marston as its first chief. During this period the researches of Marston and his colleagues covered a wide field and had important practical applications. While he always insisted that advances in practice depended on an understanding of the underlying natural phenomena, he nevertheless tried to ensure that the practical means of applying scientific principles were available. Among other subjects, the work included valuable fundamental researches on the animal's requirements for protein for the manufacture of skin and wool and on the special need for sulphur-containing amino-acids (now, but not then, common knowledge). He early observed the effect of copper on keratinization and hence on the quality of wool. Other work dealt with the requirements of the sheep for phosphorus, and of the effects on it of thyroidectomy.

In 1936 he was invited by Hopkins to spend a year in the Biochemical Laboratory, University of Cambridge. There he continued his work on carbohydrate fermentation by ruminal micro-organisms, and interested Joseph Barcroft in general problems of ruminal function, with most fruitful results.

During the Second World War he furnished, with his colleagues, valuable information to the Army on available human rations, and compiled highly useful dietary tables. After the War he worked on the basic problems of energy transactions in ruminants. The work for which he is most widely known can be traced to its beginnings in 1933, when he became interested in 'Coast disease'. Considerable stretches of land, among them the 'ninety mile desert' near his birthplace, would support neither useful crops nor domestic stock. This was (wrongly) ascribed to phosphorus deficiency. Marston and his colleagues showed that phosphorus was adequate, but that there was a lack of cobalt and copper, and in certain areas of zinc, and that traces of such elements would permit the normal growth of both plants and animals. These fundamental discoveries proved the key to the solution of difficulties encountered by agriculturists and pastoralists in other continents besides Australia, and gave impetus to the conception of the importance of heavy metals in biological processes which is still growing. Marston was able to show that cobalt was effective only after it had been incorporated into vitamin B_{12} by ruminal micro-organisms, and that at least one of its functions in the body was that of co-enzyme in certain isomeric changes. The story must now be left to other hands to unfold: that there are further chapters is certain.

Marston was widely read, and widely travelled, both in Europe and the United States, with a fund of reminiscences of people and places. In 1957 he was elected D.Sc. *honoris causa* (his first degree) by the Australian National University, and in 1959 to a D.Sc. of the University of Adelaide *ad eundem gradum*. He was invited to lecture on the work of the Division to the Royal Society of London in 1948, and was elected Fellow in 1949. In 1951, Sir Mark Oliphant and he were active in the foundation of the Australian Academy of Sciences, of which they were original members. He numbered among his close friends not only scientific colleagues such as Martin, Hopkins, Rivett, Oliphant and E. V. McCollum, but also famous artists, and leaders in the industrial world such as W. S. Robinson in Australia and J. L. Pratt and Robert Kleberg, jun., in the United States. Perhaps from such contacts sprang some of his sympathy with practical problems.

E. G. Holmes

Prof. Arthur Holmes, F.R.S.

Arthur Holmes, emeritus professor of geology and mineralogy in the University of Edinburgh, died on September 20 at the age of seventy-five at his home in Putney. His brilliant research on the wider problems of geology has earned him his place as one of the great Earth scientists of the century.

Holmes was born at Hebburn, near Newcastle-upon-Tyne, in 1890. He graduated at the Imperial College of Science and Technology in 1910 and undertook an expedition to Mozambique in 1911. Here an attack of blackwater fever nearly ended his career (there was in fact an announcement of his death in the local paper), but he came back to rejoin the staff of Imperial College until 1921. He then joined the Yomah Oil Co. in Burma as chief geologist; but, fortunately for the future of geological science, he returned in 1924 to become head of the newly created Department of Geology in the University of Durham, which under his leadership rapidly gained an international reputation for petrological research. In 1943 he was appointed regius professor of geology in the University of Edinburgh, which was honoured with his occupation of the chair for thirteen years, and with his presence as emeritus professor for six years after he retired in 1956.

Prof. Holmes has exercised a profound influence on almost all branches of geology, not only through his own remarkable researches but also by his beautifully written text-books and the stimulation of the research qualities of his students. His output of publications has been prodigious—nearly 200, including several famous text-books such as *The Age of the Earth*, *Nomenclature of Petrology*, *Petrographic Methods and Calculations* and *Principles of Physical Geology*. He has made a great number of contributions to our knowledge of the geology of many parts of the world, from his home district in the north of England to Africa, India and elsewhere; but the descriptive aspect of his work has been, far more than with most men, merely a prelude to his remarkable work on the wider genetic problems of the science. He went deeply into the major questions of the origin of igneous rocks, partly in association with his accomplished and eminent wife, Dr. Doris Reynolds. He was never a man to shun controversy, and his work on the origin of the alkaline rocks of Africa and elsewhere, on the thorny problems of granites and their associates, on the origin and development of magmas, and on the role of metasomatism in rock genesis, has broadened the vision of igneous and metamorphic petrologists all over the world.

During his school-days, Holmes's inquiring mind had questioned the magic date for the Creation, 4004 B.C., in Archbishop Ussher's chronology for the book of *Genesis*,

and in the sixth form his interest in geology was awakened by an enlightened schoolmaster who encouraged him to read Kelvin and Suess. He was thirteen years of age when Rutherford realized that radioactivity might provide a means for determining the actual ages of minerals, and he was an undergraduate at Imperial College when this idea bore fruit, and Boltwood (using lead ratios) and then Strutt (using helium content) approximately dated a number of uranium-bearing minerals. Working in Strutt's laboratory, Holmes became one of the pioneers in this field, and since that time he has been one of the leaders in applying the results of radioactive methods to the dating of rocks. He took a major part in the construction of a geological time-scale in terms of millions of years—considering both post- and pre-Cambrian rocks and the age of the Earth itself.

Holmes's major work was not confined to igneous and metamorphic petrology and geological time. His contributions to the geophysical aspects of geology are classic: the nature of orogenic and epeirogenic activity, convection currents in the Earth's mantle, continental drift, radioactivity in geology—one could go on for a long time listing the aspects of geology to which he has made major contributions. In fact there is scarcely a main branch of the subject, other than palaeontology, with which his name is not associated. Much of his thinking was on the grand scale, applied to the broad philosophical aspects of the Earth sciences.

Holmes's distinguished career received international acclaim. He was elected Corresponding, Foreign or Honorary Member of the Geological Societies of America, Belgium, and Stockholm, and the Academies of Science of the United States, Sweden. the Netherlands, and France. He was awarded the Murchison and Wollaston Medals of the Geological Society of London, the Penrose Medal of the Geological Society of America, the Fourmarier Medal of the Royal Academy of Belgium, and the Makdougall-Brisbane Prize of the Royal Society of Edinburgh. In 1964 he shared with Prof. P. Eskola of Helsinki the Vetlesen Prize of Columbia University, New York, for outstanding achievement in science relating to "a clearer understanding of the Earth, its history or its relation to the universe". He was a Fellow of the Imperial College of Science and Technology and an Honorary Doctor of Laws of the University of Edinburgh.

In spite of many years of poor health, Holmes continued his creative work until the end. The second edition (largely re-written and greatly enlarged) of his *Principles of Physical Geology*, published this year, is a masterpiece of lucid writing and shows a breadth of knowledge and vision which few men possess.

Those fortunate enough to have known Prof. Holmes found him a quiet man of extraordinary charm and unfailing kindness, and will remember with affection and gratitude the help and advice which he gave so readily. In 1914 he married Margaret Howe, and they had one son. His wife died in 1938, and he later married Dr. Doris Reynolds, herself a distinguished geologist.

F. H. Stewart

Sir Ernest Thomas Fisk

With the death of Sir Ernest Thomas Fisk at his home in Sydney on July 8, at the age of seventy-eight, the world lost a man who made a conspicuous contribution to the development of wireless communication.

Born at Sunbury-on-Thames, England, on August 8, 1886, he entered the Marconi Company's training school in 1906 and came to Australia in 1911 as resident engineer of a branch which the Company had opened in Sydney. He submitted to the Australian Government proposals for the setting up of a number of wireless stations, but his proposals were not accepted.

In the same year, the Australasian Wireless Company was formed with Australian capital to acquire and operate the patent rights of the Telefunken system. Two years later, Amalgamated Wireless (Australasia), Ltd., was inaugurated, and the rights of the Marconi and Telefunken systems were acquired. Fisk became technical and general manager, and in 1917, managing director.

In England in 1916 Fisk arranged with Marconi to conduct a series of tests, and in the following year he erected an experimental receiving station at Pymble near Sydney. Tests were conducted there with the view of determining the best type of circuit to use for long-wave, long-distance reception.

Later, Fisk transferred the station to Wahroonga, another Sydney suburb, and began experiments with the Caernarvon station in Wales early in 1918. When the tests were satisfactory, arrangements were made for a public demonstration of direct wireless communication between England and Australia.

Messages to Australian newspapers were sent from Caernarvon by the then Australian Prime Minister, Mr. W. M. Hughes, and the then Australian Minister for the Navy, Sir Joseph Cook, who were in England at that time. These, the first direct wireless messages between the two countries, were received at Fisk's Wahroonga station and delivered to the Sydney Press.

Later, the experimental receiving station was transferred to Koo-wee-rup, in Victoria, where the scope of the work was extended, new apparatus was installed, and continuous day and night observations made of signals from all high-powered stations in Europe and the United States.

The Koo-wee-rup experiments established that wireless waves from England to Australia followed the course of the greatest darkness—a discovery which ultimately was of immense value when aerials for the short-wave service were being designed.

As an outcome of successful experiments, Fisk, on behalf of Amalgamated Wireless in conjunction with the Marconi Company, in 1921 offered to establish a direct wireless service between Australia and Great Britain and to transmit messages at rates one-third less than existing cable charges. The proposal, which was strongly opposed by exponents of a relay system, found champions in Mr. W. M. Hughes and General Smuts of South Africa. They opposed the relay system and made it clear at the Imperial Conference in 1921 that their respective countries would proceed with direct communication.

Strengthened by the positive attitude of Mr. Hughes, Fisk persisted with his advocacy of a direct service, and in March 1922, on behalf of Amalgamated Wireless, signed an agreement with the Australian Government under which the Company was to establish and maintain direct wireless communication between Australia and the United Kingdom and Canada, and to assume control of existing coastal and island wireless stations. The Government acquired 50 per cent of the shares in the Company plus one to give it a bare majority, the remainder of the shares being held by private shareholders.

Fisk was not daunted by the refusal of the British Government to grant a licence to the Marconi Company to erect a high-power station in England. The experiments were continued, and in January 1924 the first transmission of short-wave signals was received from the Marconi station at Poldhu, Cornwall. In August of that year, a fresh agreement was entered into with Amalgamated Wireless to erect short-wave transmitters with directional aerials to provide service to Great Britain and to Canada when corresponding stations were built in those countries. Fisk's foresight and initiative were rewarded in 1927 with the opening of the Beam Service to the United Kingdom, and in 1928 to Canada.

Commenting that the Company had fought against powerful opposition, Royal Commissioners who investigated broadcasting within Australia reported to Federal Parliament in 1927 that the Company, Fisk and his officers were "entitled to great credit for the establish-

ment of the Beam system". They commented that ". . . it was largely due to the technical ability and persistence of its managing director that it ultimately prevailed and that Australia has the benefit of an up-to-date and extremely rapid means of communication with Great Britain. . . ".

In the field of Australian broadcasting, Fisk was a pioneer. He gave the first demonstration of wireless telephony in Australia in August 1919. Gramophone music played into a wireless transmitter was received in the lecture room of the Royal Society of New South Wales, several city blocks away. A year later he arranged a complete broadcast concert to a large audience in Parliament House, Melbourne.

He was foundation president of the Institution of Radio Engineers Australia and one of the earliest members of the Wireless Institute of Australia. He continued as chairman of directors of Amalgamated Wireless until 1945.

L. A. Hooke

NEWS and VIEWS

Royal Society Research Professorship: Prof. W. S. Fyfe

Prof. W. S. Fyfe, professor of geology in the University of California, Berkeley, has been appointed to a Royal Society research professorship. He will work at the University of Manchester and will take up his appointment in January 1966. Prof. Fyfe, who was born in New Zealand and has held appointments at the University of Otago and the University of California, is well known for his work in the field of experimental mineralogy and petrology.

Anglo-French Military Aircraft: Ministry of Aviation: Mr. J. A. Hamilton

Mr. J. A. Hamilton, formerly of the Royal Aircraft Establishment, Farnborough, has been appointed project director for the *Jaguar* strike/trainer and the variable-geometry combat aircraft to be developed jointly by the United Kingdom and France. He will be responsible within the Ministry of Aviation for the management of the two projects, acting in concert with corresponding directors in the French Direction Technique des Constructions Aeronautiques. Mr. Hamilton was educated in Scotland at Penicuik and Lasswade Secondary Schools; he graduated in engineering from the University of Edinburgh in 1943 and then joined the Marine Aircraft Experimental Establishment. He was engaged initially on the development of anti-submarine weapons and later became responsible for all flight research within the Establishment. In 1951 he joined the Aerodynamics Department of the Royal Aircraft Establishment and in 1952 he was appointed head of the Free-flight Division. During his stay at Farnborough he promoted the development of methods for conducting aerodynamic research using rocket-propelled test vehicles, eventually co-operating with the Australian Weapons Research Establishment to extend the technique into the régimes of hypersonic flight. Immediately before transfer to his present post he was head of the Projects Division of the Aerodynamics Department, and in this capacity responsible for the preliminary study of future military and civil aircraft.

Special Merit Promotions at the National Physical Laboratory: Mr. H. H. Pearcey

It has recently been announced that Mr. H. H. Pearcey, of the Aerodynamics Division, National Physical Laboratory, has had a special merit promotion to deputy chief scientific officer. Mr. Pearcey is an internationally recognized authority in several branches of fluid dynamics, particularly the interaction between shock waves and boundary layers and the many intricate features of transonic, supersonic and separated flows. He has successfully used the results of his research in the progressive development of swept-wing aircraft for cruising at high subsonic, transonic and supersonic speeds by synthesizing methods for the design of the wing section and by evolving advanced section shapes. His 'peaky' type of aerofoil, with isentropic supersonic compressions, and the work that he initiated and supervised on aerofoils with thick trailing edges, including the use of automatic ventilation to reduce the base drag, are well known. So also is his important discovery of the intrinsic connexion between the variation of static pressure at the trailing edge of a wing and the effects of shock-induced and other types of boundary-layer separation. His suggestion that this could be used to predict the threshold of aircraft buffeting has been widely exploited. Mr. Pearcey also made a classic investigation of methods for suppressing the separations that cause the buffeting and other undesirable aerodynamic characteristics. In particular, he demonstrated how the properties of vortex generators can be utilized in this respect and has been instrumental in applying them to numerous aircraft, thus effecting many significant improvements in aerodynamic behaviour and in safety at both high and low speeds. During the course of his work, Mr. Pearcey has made vital contributions to the development of experimental methods for research in high-speed flow and has played a leading part in planning, designing and commissioning the fine range of high-speed wind-tunnels now operating in the Aerodynamics Division of the National Physical Laboratory. Since 1958 he has been responsible for the High-speed Flow Group at the Laboratory; in 1963 he was awarded the Bronze Medal of the Royal Aeronautical Society, for his contributions to aerodynamics, and gained the Wolfe Award of the Department of Scientific and Industrial Research, for an outstanding contribution to the Department's research programme.

Dr. R. C. Lock

Dr. R. C. Lock, of the Aerodynamics Division, National Physical Laboratory, has been promoted to senior principal scientific officer (special merit). Dr. Lock is perhaps best known in aviation circles for his outstanding theoretical and experimental research that has produced aerodynamic design methods which render the concept of shock-free, low-drag flow—derived from the abstract infinite yawed wing—a reality for the finite-wing/fuselage combinations of practical aircraft, even for supersonic flight speeds. His papers on this subject are well established and extensively used by aircraft designers; one, on wing planform design, won the Royal Aeronautical Society's Edward Busk Memorial Prize for 1962. Dr. Lock is a leading figure in swept-wing research and plays a prominent part in several programmes in this and related fields that are co-ordinated between the National Physical Laboratory and other establishments in the United Kingdom, Europe and the Commonwealth. However, his reputation is more widely based than this. Already, before joining the Laboratory in 1954, he had had a brilliant career at Cambridge, and this had been interrupted at the end of the War by a brief but successful spell at the Royal Aircraft Establishment working on problems of aircraft design. At Cambridge he won the University Mayhew Prize for the best applied mathematics candidate in the Mathematics Tripos, Part III, and was awarded a research fellowship at Gonville and Caius College during the tenure of which he made important contributions in the fields of hydrodynamic stability and magnetohydrodynamics. At the National Physical Laboratory he first turned his attention to in-

volved problems of supersonic wing theory and wing/body interference, which led on to his work on swept wings, to his complementary experimental programme and to his active interest in the application of numerical methods and modern digital computers to outstanding problems in fluid dynamics. It is of considerable interest to note that Dr. Lock's work is in direct line of descent from the work that his father, the late C. N. H. Lock, pioneered at the National Physical Laboratory in the 1930's.

National Instrument Service in Britain

THE Science Research Council, in collaboration with the Ministry of Technology and the Office of Scientific and Technical Information, set up earlier this year a Panel under the chairmanship of Prof. G. Porter (University of Sheffield) to consider the feasibility and economics of a centralized national instrument service. A number of expensive, physicochemical instruments could provide a rapid service of routine measurements, on a repayment basis, for all scientists, such as those in Government, industrial and university laboratories. This service would enable physical-analytical techniques to be more widely available than at present and would thereby encourage the use of new instrumental techniques. Such a centralized arrangement would have additional advantages, particularly if it were associated with an interpretation service and data-store facilities. The Panel is hoping to find out whether such a service would be an effective means of meeting more economically the growing needs of research scientists for routine measurements on increasingly expensive research instruments. A description of some services which might be offered is being circulated, together with a brief questionnaire, to a cross-section of potential customers. The answers received will enable an assessment to be made of the need for services of this kind and of their economic viability. When this assessment has been thoroughly studied, the Panel will make recommendations to the Science Research Council regarding any future action.

Cambridge Engineering Freshmen

IT is too early to know the state of this year's entry of students into the technological faculties of all universities and colleges of advanced technology, but a fact which should cheer industry is that at the University of Cambridge the number of freshmen entering the Engineering Department is 304, the highest in any year since the Mechanical Sciences Tripos was established in 1892. The exceptional quality of these men is indicated by the fact that 208 of them have been admitted to the fast stream which enables them to complete the course leading to the Mechanical Sciences Tripos Part I after two years of study instead of the normal three. As recently as 1960 the rate of freshmen entering the fast stream was only about one-third of the total intake.

I.C.S.U. Bulletin

The *I.C.S.U. Bulletin* (No. 4; July 1965), issued by the International Council of Scientific Unions, contains a brief account of the third meeting of the Executive Committee of the International Council of Scientific Unions, to which was presented a report on the Scientific Committee on Oceanic Research. There has been a steady increase in the activities of this Committee, which now has twenty-seven national adhering institutions. A report was also presented from the Scientific Committee on Antarctic Research, and a discussion on the work of this Committee directed attention to the near extinction of some species in Antarctica due to efficient and intensive fishing operations, and it was suggested that the restricted areas around Antarctica should be extended from 3 to 10 miles. The Executive Committee agreed that immediate action was necessary to prevent certain whales, particularly the blue whale, from becoming extinct. It also discussed at length the increasing importance of solar terrestrial physics and the activities of the Inter-Union Commission on Solar Terrestrial Relations, its terms of reference and membership. A report was also presented from the Working Group on Relations with Developing Countries, and the suggestion was made that scientists from advanced countries should be encouraged to undertake missions in the developing countries. Scientists from the latter countries should be assisted to enable them to attend international meetings. The *Bulletin* also contains news from the scientific unions, including reports on: the work of the Committee on Atmospheric Sciences; the work of the Naples Zoological Station; the International Union of Biochemistry; and other reports from the Scientific Committee on Oceanic Research, the Committee on Space Research, the Federation of Astronomical and Geophysical Services, the Special Committee for the International Years of the Quiet Sun, and on the International Committee for Geophysics. There is also a list of publications, and the usual calendar of meetings for August 1965–January 1966.

International Paper Size

THE proposed change-over to the metric system in Britain will necessarily take a long time for full realization and it will pose many practical problems, both industrial and domestic. Not the least of these is the effect on paper sizes and printing, where tradition is likely to die very hard. If the proposed scheme of the metric-based 'International Paper Sizes' is adopted, it will completely revolutionize the present practice of the printing and publishing trades, with consequent repercussions in commerce, professional and private customs, particularly where books, pamphlets, display leaflets, stationery and envelopes are concerned, to mention only a few of the items involved. The keyword behind the conception of 'International Paper Sizes', as it is known, is simply rationalization. It means ". . . the introduction of a completely new range of paper sizes, from small to large, each based upon a metric measure and each related, in logical sequence and proportion, to the rest of the range". One result, perhaps not unwelcome, will be the replacement of the time-honoured words 'quarto', 'crown', 'octavo', etc., by a numerical code, which will be universally understood. An impartial and non-technical exposition of the definitions, advantages and disadvantages of the 'International Paper Sizes' system has recently been published in the form of a booklet entitled *Paper at Work Number 4: International Paper Sizes* (A Series of Spicers Guides. Pp. 8. London: Spicers, Ltd., 1965). Included in this booklet is a full-sized $A1$ sheet (23·4 in. × 33·1 in.), which is half the $A0$ size of 1 square metre. This $A1$ sheet is folded to demonstrate physically the full sizes of $A2$, $A3$, $A4$ and $A5$ and their relationship to each other. This example adequately illustrates the new code; the system is based on three series of sizes—all of the same proportion—designated A, B and C. The A series is perhaps the most widely used, for example, for stationery and general leaflet printing; the B series is intended primarily for larger printed items such as posters, wall-charts, etc.; the C series, in conjunction with some of the B sizes, is intended for envelopes. In the ultimate adoption of this 'International Paper Size' system there are undoubtedly benefits of cost, convenience, international standardization and simplification. The disadvantages also have to be faced, but in the long run should not prove insuperable; for example, some printers' machinery may not be geared to handle 'International Paper Size' with optimum productivity; also office filing, filing equipment, addressing machines, etc., may not be suitable for dealing with 'International Paper Size' sizes; but these hazards will be overcome eventually, and meanwhile everybody concerned has time for consideration and thoughtful reappraisal of the important

issues involved. Finally, the vital concern of all industry in Britain at the present time is export and it is here that 'International Paper Size' has come into its own. Twenty-six countries are using the system for commercial and technical paperwork; these include Austria, Belgium, Denmark, Finland, Germany, Holland, Italy, Norway, Portugal, Spain, Sweden and Switzerland.

Millboard Manufacture

THE main raw material in the manufacture of millboard is waste paper, and this, like many of the other commodities used in the process, has considerably increased in price since the end of 1964. Coupled with increased wages and overheads, and the fact that many overtime hours have had to be worked in the factory at Bourne End, Bucks., due to permanent under-staffing, the price structure of the products of Jackson's Millboard and Fibre Co., Ltd., has been correspondingly increased. These and some other interesting items are disclosed in the statement of the chairman (Mr. M. W. Jackson) at the seventy-fourth annual general meeting on September 29, 1965 (*Jackson's Millboard and Fibre Co., Ltd., Directors' Report and Accounts, 1965*. Pp. 12. London: Jackson's Millboard and Fibre Co., Ltd., 1965). The first fully automated intermittent board-making machine in Britain was installed in the Company's main factory in 1962. In this connexion, Mr. Jackson says: "In my report in September 1964 I referred to the purchase of the board making unit from Norway, and this installation is now on the point of completion. We have also modernized one of our existing board-making units at present concerned with the production of leatherboard, and this makes our second automatic machine. It is hoped that well before the end of the year we shall have three automatic board-making units in operation at Bourne End". These innovations should help considerably to mitigate price increases and expand the Company's export trade, an important item. But in spite of all efforts to improve efficiency, including expenditure on modern plant as described, ". . . the increases in operating costs over recent months are more than we are able to bear, if we are to show a reasonable return". Thus, even an advanced state of automation in manufacture, such as is in process of achievement in this factory, would not appear to be a panacea for all the economic ills in this industry at the present time. It can only be hoped that Mr. Jackson's somewhat gloomy foreboding will be dispelled by events this year in the shape of increased turnover and export business, especially in the Far East, where interesting results of overseas visits appear already to be bearing fruit.

Particle Size Measurement

A REPORT of a meeting, held in November 1963, at the British Ceramic Research Association, Stoke-on-Trent, to consider particle size measurement, has recently been published by the British Ceramic Society on behalf of the European Ceramic Association (Pp. 20. British Ceramic Society, Shelton House, Stoke-on-Trent, August 1965. 5*s*.). Measurement of particle size and interpretation of results to practical advantage are common problems in the ceramic and many other industries. Recent years have seen many improvements in traditional methods of measurement, such as using the microscope and the technique of sedimentation, but methods based on new principles have also appeared. It is pointed out, however, that unfortunately none of the latter has universal application, and so diversity of method increases. At the meeting four prepared contributions formed a basis for discussion: a review of the purpose of analysis and problems involved as seen by the British Ceramic Research Association; a report on a recent classification of methods undertaken by a sub-committee of the Society for Analytical Chemistry; an assessment of the Coulter counter as a means of analysis of some ceramic materials; and an account of size analysis of clays within the submicron range. On the subject of appraisal of methods of particle size measurement now commonly publicized, some interesting results are revealed from replies to a questionnaire sent to industrial concerns, universities and colleges of technology. The questions concerned methods actually in use, type of material analysed, size-range, purpose of measurement (routine control, research, etc.). In so far as replies from 69 respondents may at this stage be considered representative, cautiously interpreted, they are certainly indicative, and fall into line with what might well be anticipated as the trend of things to-day. Summarily, sieving is the most frequently used single method; the Andreasen pipette method follows closely; sedimentation methods are generally popular; elutriation is relatively seldom used; graticules are frequently used, so also is the Coulter counter, while permeability methods receive a fair share of support. Of the 74 methods of particle size measurement in use and listed in the classification, 24 were not used by the respondents. Of the 69 replies received, 32 respondents used one method or another in routine control operations, 61 in research or development work. It is planned to organize comparative tests of some of the better-known methods on a large scale; possibly this may take the form of analysis by different laboratories of the same sample by the same method, or the analysis by the same laboratory of the same sample by different methods.

Surface Active Agents

SURFACE active agents are playing an increasingly important part in the manufacture of many diverse products in use in everyday life. These vary from materials such as oil paints and plastics, to duplicating inks and deodorants. These agents are incorporated in industrial and domestic preparations not only for their 'point of use' effect, for example, in shampoos, paint removers, etc., but also to facilitate manufacture, as in the case of emulsion paints, or to promote consistency in products such as cosmetic creams. The problem of determining the type of surface active agent present in a variety of different preparations is one constantly facing many analytical and industrial chemists, some of whom, by long experience, are experts in this field. Others, however, possibly the majority, have limited experience of these agents, and the apparatus requisite to their analysis. For the latter group, particularly, a most useful handbook has recently been published entitled *Surface Active Agents: their Extraction, Characterisation and Determination*, by Maurice Bell (Pp. 24. Leeds: Glovers (Chemicals), Ltd., 1965. 5*s*.). It is shown in this booklet that, in spite of the wide range of products of which surface active agents are important constituents, it is possible to divide such materials into a comparatively small number of groups: emulsions (oil-in-water or water-in-oil); aqueous based (that is, dissolved in water or mixtures of water and water-miscible solvents); non-aqueous based (dissolved or dispersed in oils, fats or organic solvents); and dry based (adsorbed on or admixed with powdered inorganic or organic materials, or similar in tableted or cake form). With this classification in mind, the various techniques and tests are considered under the appropriate headings: separation of the isolated surface active agents; characterization of the isolated surface active agents; quantitative examination of surface active agents; control of surface active agent concentration; chemical methods of control. The directions throughout, although to some extent summarized, are clear, concise and practical; if further detailed information on any procedure is required, then the list of references included will help. Domestic soap and detergent powders, washing-up liquids, and the like, are excluded from this booklet. They are the subject of special investigations and analytical schemes described

by other authors, to whom appropriate references are quoted. This little monograph is to be commended. It is the first of a series which it is the intention of the publishers to issue from time to time on various aspects of surface chemistry.

A Fresh Approach to the Problem of Allergy

G. B. West and J. M. Harris of the Department of Pharmacology, School of Pharmacy, University of London, have shown that rats secured from one Wistar albino colony are resistant to the first injection of dextran or egg-white, and that they fail to develop gross oedema of the extremities (the so-called anaphylactoid reaction) (*Ann. N.Y. Acad. Sci.*, 118, Article 8: *Pharmacogenetics —a Fresh Approach to the Problem of Allergy*. Pp. 439—452. New York: New York Academy of Sciences, 1964). Selective breeding experiments showed, first, that this non-reactivity is an autosomal recessive character, and secondly, that it is not linked with the colour genes for black or yellow. Pure stocks of non-reactor rats were established for both albino and non-albino strains, confirming that the anaphylactoid reaction is genetically controlled. Egg-white allergy in man is also partly inherited and also produces severe oedema of the exposed parts. It will be important, therefore, to determine the cause of non-reactivity of rats to egg-white and dextran, particularly as some genetical polymorphisms in man are known to produce biochemical differences.

Soil and Landscape at Archaeological Sites

It is not unusual for the pedologist to be concerned in the study of former occupation sites, and an interesting paper on the examination of soil profiles, and a suggested chronology of events in such a neighbourhood, has been prepared by P. H. Walker (*Records of the Australian Museum*, 26, No. 7; June 12, 1964: *Soil and Landscape History in the Vicinity of Archaeological Sites at Glen Davis, New South Wales*. Pp. 247–264+plates 25–27. Sydney: The Australian Museum, 1964. 6*s*.). The sites are in shelters formed from large erratic sandstone boulders on the lower slopes of the Capertree River Valley, Australia, where they are characterized by several hundred feet of intensively weathered coarse sandstone talus with narrow terraces. The debris on the upslope side of boulders reveals the past movement of the superficial deposits. The country rock is highly quartzose Triassic sandstone displaying strong current bedding. The deposits of the cave floors are relatively uniform and unstratified, and contain few rock fragments in comparison with the soil outside; but, according to modal particle size, are derived from the sandstone rock, are less sorted than the river deposits and have arisen from the deposition of coarse material in a sheltered position. Artefacts are found in the loose, sandy layers of the soil, which is unique in the locality. The soils inside and outside the caves are, however, to be regarded as minor surface disturbances of short duration in the development of the valley.

Gairdner Foundation Awards

The 1965 Gairdner Foundation annual awards, valued at 5,000 dollars, have recently been awarded to: Dr. Charles P. LeBlond, head of the Department of Anatomy, McGill University, Montreal, for development of the technique of autoradiography; Dr. Jerome W. Conn, University Hospital, Ann Arbor, Michigan, for his pioneering work on the clinical significance of aldosterone; Dr. R. R. A. Coombs, Department of Pathology, University of Cambridge, for his work on antibody on the surface of red blood cells; Dr. Charles E. Dent, University College Hospital, London, for his work on paper chromatography leading to the discovery of diseases caused by imbalance of amino-acids; Dr. Daniel J. McCarty, Hahnemann Medical College and Hospital, Philadelphia, for his work on the causes of gout and rheumatoid arthritis; Sir Horace Smirk, University of Otago Medical School, Dunedin, New Zealand, for his work on the application of nerve-blocking drugs in the treatment of high blood pressure. The Foundation was established in 1957 by J. A. Gairdner, the Toronto industrialist. Annual awards of 5,000 dollars are bestowed on those who "have made contributions to the conquest of disease and the relief of human suffering".

David Anderson-Berry Prize, 1966

A David Anderson-Berry Medal, together with a sum of money, will be awarded by the Council of the Royal Society of Edinburgh during 1966, for recent work on the effects of X-rays and other forms of radiation on living tissues. Applications and proposals on behalf of others should be addressed to the General Secretary, Royal Society of Edinburgh, 22–24 George Street, Edinburgh 2, from whom further information can be obtained. The closing date for applications will be March 31, 1966.

Announcements

Prof. O. V. S. Heath, at present professor of horticulture in the University of Reading and honorary director of the Unit of Flower Crop Physiology of the Agricultural Research Council, has been appointed to fill a vacancy in the membership of the Agricultural Research Council, which has arisen on the retirement, after ten years' service, of Sir Hans Krebs.

The sixth seminar on "Electrochemistry" will be held at the Central Electrochemical Research Institute, Karaikudi, during December 26–29. Further information can be obtained from Dr. K. S. Rajagopalan, Central Electrochemical Research Institute, Karaikudi 3, Madras State.

Several oceanographic meetings and conferences, sponsored by the Institute of Marine Science, will be held at Miami Beach during November 11–24. Further information can be obtained from the Institute of Marine Science, University of Miami, 1 Rickenbacker Causeway, Miami 49, Florida.

An international conference on "Radiological Protection in the Industrial Use of Radioisotopes", organized by the Société Française de Radioprotection, will be held in Paris during December 13–15. Further information can be obtained from the Secretary General, Société Française de Radioprotection, Boîte Postale 3, Fontenay aux Roses, Seine.

The winter meeting of the British Biophysical Society, on "The Conformation of Biological Macromolecules", will be held at Queen Elizabeth College, University of London, during December 20–22. Further information can be obtained from Prof. R. E. Burge, Department of Physics, Queen Elizabeth College, Campden Hill Road, London, W.8.

The International Institute for Conservation is planning to hold a conference on "Chemicals in Museums: Quality, Care and Safe-handling" at the Institute of Archaeology, in the University of London, on January 27, 1966. The speakers invited to take part will include a factory inspector, the medical adviser to the Trades Union Congress, a representative from the firm of Hopkin and Williams, and a member of the Government Laboratory. Further information can be obtained from Miss Gedye, Institute of Archaeology, 31 Gordon Street, London, W.1.

An international conference on the "Lymphatic System", sponsored jointly by the Committee on Shock of the National Academy of Sciences–National Research Council and the Tulane University School of Medicine, will be held in New Orleans during December 12–14. Further information can be obtained from Prof. H. S. Mayerson, Department of Physiology, School of Medicine, Tulane University, New Orleans.

USE OF PIGS IN BIOMEDICAL RESEARCH

By Dr. L. K. BUSTAD
Biology Department, Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington
AND
R. O. McCLELLAN
Medical Research Branch, Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington, D.C.

THE first symposium devoted exclusively to the basis for and extent of utilization of pigs in biomedical research was held at the Pacific Northwest Laboratory, Richland, Washington, during July 19–21, under the sponsorship of the U.S. Atomic Energy Commission and the Battelle Memorial Institute (contract *AT*(45–1)1830). About 150 scientists from Canada, Denmark, France, Germany, Great Britain, Switzerland and the United States joined scientists of Battelle Memorial Institute's Pacific Northwest Laboratory (a U.S. Atomic Energy Commission facility formerly operated as the Hanford Laboratories by the General Electric Co.) for three days of formal presentations and panel discussions.

The symposium was divided into four general categories: (I) comparative anatomical and physiological characteristics; (II) biomedical applications of pigs and miniature pigs; (III) laboratory management practices; (IV) miniature pig development programmes. Session and panel chairmen were: D. L. Anderson, Division of Biology and Medicine, U.S. Atomic Energy Commission; T. J. Cunha, University of Florida; D. K. Detweiler, University of Pennsylvania; H. W. Dunne, Pennsylvania State University; D. C. England, Oregon State University; T. Gillman, Institute of Animal Physiology, Babraham, Cambridge; W. Montagna, Oregon Regional Primate Research Center; L. E. Mount, Institute of Animal Physiology, Babraham, Cambridge; and J. H. Rust, University of Chicago.

D. F. Cox, Iowa State University, reviewed pig genetics, pointing out that with the exception of information on the inheritance of blood antigens of pigs, knowledge of the genetics of inherited traits which are controlled by a few genetic factors is limited. He noted that domestic pigs provide some unique opportunities for research because of the vast array of genetic variation both between and within the various breeds.

Inheritance investigations described by J. Moustgaard and M. Hesselholt, Royal Veterinary and Agriculture College, Copenhagen, have shown that the presence or absence of antigenic factors on the surface of pig erythrocytes is genetically controlled by allele genes belonging to fourteen chromosomal loci. Blood group systems have been established and designated by letters A B C E F G H I J K L M N and O.

R. A. McFeely, University of Pennsylvania, presented recent work on pig cytogenetics. The pig appears particularly well suited for cytogenetic investigations because it has only 38 chromosomes, which can be readily paired and grouped in a manner not unlike human chromosomes. Of particular interest are studies in pigs with intersex conditions where chromosome analyses in several cases have shown that the role of the *Y* chromosome in the determination of sex may be somewhat different from that in man and the mouse.

D. Smidt *et al.*, University of Göttingen, reported on their investigations of reproduction using miniature pigs. They obtained fertilized ova by flushing oviducts and transferred the eggs from miniature sows to other miniature sows and reciprocally between miniature and Landrace sows. The embryo implantation rate was 25 per cent, and 50 per cent of these were carried to term. The size of the sow influenced the birth weight and the proportions of the pigs. The weight differential persisted until they were about 6 weeks old.

Birthe Palludan, Royal Veterinary and Agricultural College, Copenhagen, presented an interesting, comprehensive review of teratological studies in pigs with avitaminosis-A. A number of malformations were observed, the most frequent being microphthalmia. No malformations were seen if vitamin A was administered to vitamin A-deficient sows before the 16th–17th day of gestation. Administration of large doses of vitamin A to pregnant sows caused eye anomalies and malformations of the heart similar to those caused by vitamin A deficiency. She also observed that thalidomide administered at high levels to pregnant pigs caused retarded development of the palate, heart, lungs, alimentary tract, and urogenital system.

L. E. Mount, Institute of Animal Physiology, Cambridge, described the patterns of heat production and heat loss in the new-born pig, relative to the pig's environment, and discussed some similarities to those observed in the human new-born.

S. L. Hansard, Louisiana State University, described extensive investigations of placental transfer and foetal utilization of absorbed minerals. He noted that absorbed minerals passed freely from dam to foetus at a rate which was inversely proportional to ionic size, but that the cations iron, calcium, phosphorus, and zinc traversed the placenta more rapidly than the anions iodine and phosphorus.

Several papers described work in the areas of dental and skeletal research. E. B. Jump and M. E. Weaver, University of Oregon Dental School, who pioneered the use of miniature pigs in dental research, described some advantages and limitations of this species. They noted that the pig masticates with both incision and trituration and is unique among the common laboratory animals in having a long period of deciduous and transitional dentition. This latter feature permits experimental investigations on many dental problems afflicting children. Their preliminary work demonstrates the suitability of miniature pigs for clinical experiments in restorative dentistry, orthodontics, periodontics, and the pathology and therapeutics of the tooth pulp. An interesting, but unexplained, difference they noted between man and pig was that the latter produces larger amounts of dental calculi without experiencing the periodontal pathology associated with dental calculosis in man.

A paper by H. D. Dellmann and R. C. McClure, University of Missouri, described skull measurements to establish a co-ordinate system for stereotaxic placement in the brain of the pig. Their results suggest the possibility of developing a stereotaxic atlas for pigs even though breed and strain differences exist.

F. A. Spurrell, University of Minnesota, presented results obtained by him and his associates, W. J. L. Felts and L. A. Baudin, on the development of osteons in

pig and man, and the effect of dietary calcium restrictions. Cortical bone development in miniature pigs was compared and found similar to that in the standard domestic pig.

The structure of bones of starved pigs was evaluated by C. W. M. Pratt in a paper he presented with R. A. McCance, University of Cambridge. Newly weaned pigs were subjected to severe undernourishment for periods up to 1 year, maintaining their weight at 3–8 kg.

Their bones, although showing no specific pathology, were structurally distinctive, being unlike either normal growing bone or miniature mature bone. On the basis of calcium to collagen ratio, the bone appeared to be hypercalcified. Recovery of these undernourished animals occurred when they were allowed unlimited food. All bones ultimately reached close to their expected length, although mild deformities of the shaft appeared in some bones.

Only one paper on renal physiology was presented. In this paper, T. W. Nielsen with C. A. Maaske, University of Colorado, and N. H. Booth, Colorado State University, reported on their work on standard renal function tests performed on conscious unanaesthetized pigs. Endogenous creatinine, insulin, PAH, and osmotic clearances were determined. They reported that the pig has predominantly short-looped nephrons (97 per cent), compared with 86 per cent for man, whereas in the dog nearly 100 per cent of the nephrons are long. The ability of the pig to concentrate urine was also comparable to man. The pig, however, appeared to be insensitive to urea loading, and acetylation of the PAH in the pig probably compromises its use for clearance measurements using this material.

Several papers discussed gastrointestinal function and nutrition. D. F. Magee, University of Washington School of Medicine, described investigations of pancreatic secretion in pigs, which he found to have several advantages over dogs. He found that the volume of juice secreted is directly related to the hydrogen ion concentration in the duodenum of *p*H 7–1 and (unlike the dog) peptone, oleic acid and olive oil all increased the output of amylase and lipase in the juice. Above *p*H 7, inhibition of secretion was noted. The pancreas seems to be involved in the regulation of duodenal *p*H on both sides of neutrality.

In a paper by T. W. Perry *et al.*, Purdue University, oesophago-gastric ulcers observed sporadically in pigs were described and were considered similar to those found in man. A high incidence of these ulcers could be produced by feeding corn, gelatinized corn, wheat, barley, or oats. Dietary additives such as α-tocopherol, menadione, vitamin A, antibiotics, copper, and methionine were without effect in preventing the oesophago-gastric ulcers.

W. G. Huber and R. F. Wallin, University of Illinois, analysed the gastric secretion from pigs bearing Heidenhain pouches or simple gastric fistulae. Differences were noted in *p*H, free acid, chloride, pepsin and histamine, between the two types of surgically prepared pigs; however, no significant difference was observed in total acid secretion. Difficulty was encountered in maintaining electrolyte balance in the pigs with the Heidenhain pouches.

Work on the sex-related differences in body composition in the pig and human infant was described by L. J. Filer *et al.*, University of Iowa. Filer noted that information on body composition of human infants is almost entirely restricted to that which may be accumulated by indirect methods of examination. He proposed that the growing pig be used as a model for comparisons to the human infant, permitting investigations on the influence of age, sex and diet on the rate of growth and body composition during infancy.

The application of pigs in investigations of severe protein malnutrition was discussed by W. G. Pond *et al.*, Cornell University. They found that when pigs were weaned at 3 weeks of age to dry, low-protein diets, biochemical and anatomical changes occurred which resembled kwashiorkor in human infants. When a 3 per cent protein, low-fat diet was fed, the pigs remained active and alert and failed to develop the severe liver pathology and oedema seen in pigs fed a 3 per cent protein, high-fat diet.

B. C. Johnson, University of Illinois, also reported on the results of undernutrition of pigs, with emphasis on enzymatic and cardiovascular effects of starvation and re-feeding. Apparent irreversible damage was produced in myocardium as well as arteries and arterioles as a result of stresses of re-feeding following starvation. Johnson noted diastolic hypertension after only two starvation–re-feeding episodes.

E. R. Miller and D. E. Ullrey described cardiovascular effects in baby pigs with dietary deficiencies. Thiamine-deficient pigs consistently exhibited bradycardia, sinus arrhythmia, and atrioventricular blocks. In recent years, pigs have been increasingly used in cardiovascular research. In deference to this widespread interest, an evening panel was devoted entirely to cardiovascular research. D. K. Detweiler, University of Pennsylvania, who chaired this panel, reviewed the growing utilization of pigs in cardiovascular investigations, especially in North America and Germany. W. von Engelhardt, School of Veterinary Medicine, Hanover, presented a comprehensive review of pig cardiovascular physiology.

H. Luginbuhl, a Swiss scientist now at the University of Pennsylvania, discussed spontaneous atherosclerosis in pigs. Although it has been known for a decade that pigs develop lesions comparable to those of the pre-atheromatous phase in human atherosclerosis, Luginbuhl is the first to report on a large series of aged pigs (ranging from 8 to 14 years old). In these pigs, pre-atheromatous changes progressed to the formation of atheroma in the aorta, iliac, cerebral and coronary arteries of several animals. Luginbuhl identified all tissue elements constituting atheroma of man, and described for the first time the detection of complicated atherosclerotic lesions with ulceration and thrombus formation in pigs. Areas of cerebral occlusion were comparable to those in man.

H. C. Rowsell *et al.*, Ontario Veterinary College, reported on some of their investigations of the effects of dietary substances found in the diet of man on the development of atherosclerosis in pigs. Their work has shown that diets high in butter, egg yolk and lard plus cholesterol increased the amount of atherosclerosis and thrombosis in pigs. Rowsell *et al.* observed that, in endothelial preparations from pigs as young as 2 weeks of age, deposits rich in platelets occurred around vessel orifices and bifurcations in a topography and pattern similar to early atherosclerosis. Pigs were found to be ideally suited for the examination of the interactions of the vessel wall, the blood platelet, and blood coagulation.

Several papers were devoted to experiments involving heart surgery. C. A. Maaske, together with N. H. Booth and T. W. Nielsen, University of Colorado and Colorado State University, described experimentally induced cardiac failure in pigs. By partially occluding the main pulmonary artery of the pig, they produced a slow, progressive, right-sided, congestive heart failure accompanied by signs of clinical heart failure. They noted that induction of congestive heart failure in pigs was particularly significant, since cardiac failure was induced by a single surgical manoeuvre, while in dogs, multiple assaults or series of surgical manoeuvres are required to produce comparable results. At necropsy, grossly distended central veins, right atrial and ventricular dilatation and hypertrophy, hepatomegaly, and ascites were observed in affected animals.

G. D. Lumb, Warner-Lambert Research Institute, Canada, also reported on investigations of experimentally induced cardiac failure in pigs. Taking advantage of the similarity of the distribution of the coronary arteries of pig and man, he ligated the coronary branches supplying

the atrioventricular node and bundle of His, and then examined the degree of collateral circulation that ensued and the beneficial effects of medication.

D. C. Sawyer and H. L. Stone, U.S. Air Force School of Aerospace Medicine, reported on techniques and results of thoracic surgery for implantation of sensing devices for monitoring cardiac output and other parameters. They presented a coloured film on their surgical technique for implanting electromagnetic flow sensors placed around the ascending aorta. Implants remained functional more than 40 days. They reported values of 5·3 l./min for cardiac output, a stroke volume of 41 cm³/beat and a heart-rate of 124 beats/min in unanaesthetized pigs trained to recline quietly on a laboratory table. The major reason for their choice of miniature pigs over the dog for these investigations was the high incidence of aortic rupture in surgically prepared dogs.

In a more fundamental investigation, N. H. Booth with H. E. Bredeck and R. A. Herin, Colorado State University, reported on their work on the baro- and chemo-receptor reflex mechanism in pigs. H. Hoernicke, University of Hanover, closed the cardiovascular panel by reviewing the Berlin symposium on circulation in pigs. His review was largely concerned with *hertztod*, a cardiac disease in pigs of considerable economic importance in Germany. Differences in cardiovascular performance between domestic and wild pigs were also discussed. He noted that body-temperature loss appears to be an important factor in exercise tolerance by domestic pigs.

Normal haematological and biochemical parameters of miniature pigs were described by A. S. Tegeris *et al.*, Food and Drug Administration, and by R. O. McClellan *et al.*, Battelle-Northwest. Their papers provided extensive information on more than 30 parameters. Both papers emphasized the need for dynamic investigations in evaluating haematological and biochemical changes. McClellan emphasized the dynamic changes which appear to be age-related.

Another very interesting application of pigs is in certain immunological investigations, for which they seem uniquely suited. D. Segre, University of Illinois, stated that the immunological incompetence of colostrum-deprived baby pigs could be overcome by the administration of the antigen mixed with minute amounts of specific antibody or with large quantities of normal γ-globulin. By such investigations, support was generated for the natural selection theory of antibody formation, which holds that the antigen–antibody complex rather than the antigen alone constitutes the proper antigenic stimulus. The results presented by Y. B. Kim and by D. W. Watson differed somewhat from those of Segre. These workers, together with S. G. Bradley, University of Minnesota, found that "immunologic virgin" pigs appear to be immunologically competent, as manifested by their excellent response to antigenic stimuli—a single intraperitoneal injection of 10^{12} particles of actinophage *MSP*-2. They found that germ-free, colostrum-deprived miniature piglets taken by hysterectomy 3–5 days before term were free of detectable immunoglobulins and antibodies if great care was exercised in preventing contamination of the new-born pig, with any foreign contaminant such as dam's blood. Of related interest also to immunologists was a paper by D. E. Ullrey, C. H. Long and E. R. Miller, Michigan State University, in which they investigated the absorption of intact proteins from the first milk of the mother for protection of the new-born pig. Colostrum-deprived pigs were used to establish that intact protein could be absorbed from the intestine after birth. This was done by feeding either a protein-free or protein-containing purified diet labelled with fluorescein isothiocyanate. One of their interests is to determine the mechanism of "gut closure", and in this regard they suggested that we may not have to wait very long for the clarification of this mechanism. If means for readily 'opening' and 'closing' the gut to γ-globulin-size particles could be developed, it would represent a significant fundamental and applied technique for those interested in areas such as immunization and anaphylaxis.

Standard pigs are commonly afflicted with an arthritic condition that is of economic concern to pig producers. G. M. Neher and J. M. Carter, Purdue University, suggested that this naturally occurring arthritis of pigs bears many similarities to rheumatoid arthritis in man, although the aetiological agent is not common to both species. They observed that the disease could become active at any stage, and complete remissions were common in the early stages. Their work suggested that hypersensitivity is involved in the aetiology of the disease. R. W. Moore pointed out that at Texas A and M they have produced a mycoplasm arthritis in pigs which may be a superior model for rheumatoid arthritis in man.

F. D. Klopfer related the results of some of his investigations of visual learning in pigs at Washington State University. He found that unless special procedures are used in rearing very young pigs, they do not learn readily to solve visual discrimination problems for food reward. The effective procedures appear to be those preventing the strong development of position responses in feeding, so as to permit the development of visual discrimination while feeding. Using discrimination learning procedures, Klopfer found it was possible to demonstrate wave-length discrimination in these animals and to determine the photopic and scotopic visibility functions.

Pigs have been used quite extensively in radiobiological investigations; examples of present research in this area were reviewed in several papers. U.S. Naval Radiological Defense Laboratory scientists, in a paper presented by N. P. Page, described the recovery pattern of pigs given a large sub-lethal dose of X-irradiation. They found the acute $LD_{50/30}$ (mid-line air dose) of 8- to 9-month-old gilts was about 400 r. for 1 mVp. X-rays. Recovery from the effects of 238–265 r. exposures was estimated by redetermining the LD_{50} at various times after their initial exposure. The animals had recovered from 48 per cent of the initial injury by 3 days, and by 7 days the majority of animals appeared to have recovered completely. In fact, the data suggested that some of the animals had become 'radio-resistant'. By 20 days the LD_{50} was about 170 per cent of the LD_{50} of the unconditioned animals.

In another paper on the effects of external whole-body radiation, D. G. Brown, University of Tennessee Agriculture Research Laboratory, reported on investigations of the late effects of pigs exposed to 15–700 rads of mixed neutron γ-radiation from a nuclear detonation 8 years ago. About 44 per cent of the irradiated pigs still survive compared with 69 per cent of the controls. The increased mortality in the irradiated pigs is attributed to gastrointestinal alteration and neoplasia.

R. O. McClellan reviewed the use of pigs in radionuclide toxicity investigations describing long-term examinations of the effects of daily ingestion of radiostrontium, investigations of the gastrointestinal absorption of plutonium and SNAP radionuclides, and metabolism and effects of plutonium deposited in the skin. He emphasized the importance of the size of the miniature pig, gastrointestinal tract and relatively long life-span as pertinent factors in favouring the use of the pig because of the importance of these criteria when results may be extrapolated to man. Of special interest was the reported high incidence of haematopoietic tissue neoplasms in miniature pigs ingesting large quantities of strontium-90.

For many years workers have regarded the skin of pigs as being very similar to human skin; it is only recently that careful, thorough, anatomical assessment has been accomplished by one of the outstanding authorities on skin, William Montagna. In a brief review of his work, Montagna stated that although pig skin shares some anatomical and histochemical features with that of man, it is distinctly different. The skin of both is characterized

by a sparse hair coat, a thick epidermis with a well-differentiated under-sculpture, a dermis which has a well-differentiated papillary body, and most noteworthy, a large elastic tissue content. In contrast to man, the pig dermis is poorly vascularized and the sebaceous glands contain much alkaline phosphatase. In view of his observations, Montagna recommended that caution should be exercised in suggesting that there is a strong resemblance between the two skins.

In his interesting paper on pig skin, G. D. Weinstein, University of Miami, found the kinetics of epidermal proliferation to be similar in man and pig. Using tritiated thymidine labelling, Weinstein showed that labelled cells in the epidermal basal layer had a transit time through the viable epidermis of 14 and 13 days in the pig and human epidermis, respectively. The total turn-over time of pig epidermis is about 30 days, while in human epidermis it is 27 or 28 days.

T. Gillman presented a film describing work on histogenesis of healing incised and excised wounds which he has performed with his associate, L. Ordman, at the Institute of Animal Physiology, Cambridge. Young pigs have been his principal subjects. Gillman stressed the indispensability of three-dimensional analysis of histogenesis. Sutures, he noted, evoked marked reactions especially in epidermis and skin appendages transected by the suture needle. Tape closure utilizing micropore (Minnesota Mining and Manufacturing Co.) was found highly effective in eliminating these complications and in permitting perfect healing without wound disruption. The results of this work demonstrate that widely accepted descriptions of healing cutaneous wounds are incorrect and therefore require revision.

Two papers described the results of radiation exposure of pig skin. In the first paper, J. O. Archambeau *et al.*, Brookhaven National Laboratory, reported on the use of pig skin for evaluating the effects of ionizing radiations. They concluded that the histology and radiation geometry of pig skin resembled the human sufficiently to warrant its use as an *in vivo* system for comparing effects of different types of ionizing radiations and extrapolating these results to man.

In a paper by the late L. A. George and L. K. Bustad, Battelle-Northwest, gross observations of early and late changes in pig, sheep and rabbit skin after acute exposures to phosphorus-32 or strontium-90 plaques were described. The pattern of early response observed in pigs resembled generally that described for man.

One day of the symposium was set aside for panel discussion of laboratory management and miniature pig development. L. E. Mount, Institute of Animal Physiology, Cambridge, was chairman of the panel on housing and handling. The members of this panel were: D. F. Cox, Iowa State University; A. Dettmers, University of Minnesota; F. L. Earl, Food and Drug Administration; R. L. Murphree, University of Tennessee (AEC); and R. O. McClellan, U.S. Atomic Energy Commission. The panel reviewed experimental pig units at their sites, and in this connexion made recommendations on housing, including floor space, air movement, temperature, feeding, water, and farrowing facilities, and on techniques in handling, including bleeding and anaesthesia.

S. G. Hastings, N. H. Booth and M. L. Hopwood, Colorado State University, submitted a paper on general anaesthesia for thoracic surgery procedures, and G. L. Waxler and C. K. Whitehair, Michigan State University, read a paper on techniques for using germ-free pigs in biomedical research. J. J. Landy, University of Miami, gave a brief report on his work with delivery and maintenance of germ-free pigs and the application of these procedures in human surgery.

Another panel discussed nutrition of pigs. The panel included the chairman, T. J. Cunha, University of Florida; A. Dettmers, University of Minnesota; L. K. Bustad for V. G. Horstman, Battelle-Northwest; L. J. Filer, Ross Laboratories; E. G. Hill, University of Minnesota; C. K. Whitehair, Michigan State University; and J. P. Bowland, University of Alberta.

In addition, E. G. Hill submitted a paper on fatty-acid composition of miniature pig tissue lipids, and J. P. Bowland submitted a review of his extensive work on the composition of pig milk.

The third panel discussion was on disease and disease control. The chairman of the panel was H. W. Dunne, Pennsylvania State University. The panel members were: D. G. Brown, University of Tennessee (AEC); F. L. Earl, Food and Drug Administration; A. G. Edward, University of California, Davis; R. A. Griesemer, Ohio State University; R. O. McClellan, U.S. Atomic Energy Commission, for H. A. Ragan, of Battelle-Northwest; and M. J. Twiehaus, University of Nebraska. The panel reviewed the disease problems encountered and most likely to be encountered in experimental pig herds, and recommended an effective disease control programme for experimental pig herds and for the use of pigs in the laboratory.

D. C. England, Oregon State University, was the chairman of a panel on miniature pig development. The panel members were: W. E. Rempel, University of Minnesota; L. Welch, University of Nebraska; D. Smidt, Göttingen, Germany; J. C. Taylor, U.S. Department of Agriculture; P. Nizza, Fontenay-aux-Roses (Seine), France; and L. K. Bustad, Battelle-Northwest. The panel discussed the concept of miniature pig development and implementation of the concept, and defined essential features of existing herds.

In his introductory statement, D. C. England recalled that in 1949 the Hormel Institute of the University of Minnesota began the first project to develop a breed of miniature pigs specifically for use in biomedical research. Medical personnel of the Mayo Foundation gave added impetus, since it recognized the need for a convenient experimental animal that would better meet certain anatomical and physiological demands. The objectives of the University of Minnesota programme were to develop pigs with a mature size small enough to be easily handled and maintained, with normal physiological characteristics.

In the review of the various herds under development it was clear that one had an assortment of strains from which to choose. Several herds are white-skinned and have adult body-weights about the size of man.

An interesting recent introduction of the Labco pig, a gentle, sparsely haired pig obtained from Mexico by H. Quick, of Homewood, Illinois, into Hanford miniature pig was demonstrated. Many of the offspring were white and had sparse hair coats. The 'Labco' miniature and the 'Vitavet' lab. miniature developed by F. Conder, of Marion, Indiana, represent the only private development of miniature pigs which have come to our attention.

A promise of some very small stock from Nepal was reviewed by W. Rempel, University of Minnesota, who showed pictures of some pigs that weighed only about 10 lb. at one year of age. This is even smaller than the Vietnamese pigs which were imported by Haring *et al.* into Göttingen, Germany, and crossed with German 'Landrace' and 'Hormel' miniatures in their laboratory.

It appears that two classes of miniature pigs will be established on the basis of predicted need. Not only is there a real demand for pigs which have an adult weight less than 30 kg, but also for one that as an adult weighs about 70 kg, similar to the so-called standard man and to the weight of most of the miniature pigs now being used.

Collectively, the papers presented at the symposium reflect the present state of our knowledge on the applicability of pigs in biomedical research and establish the pig as an important and very promising experimental animal.

With the development of a standard 60-lb. pig which may well be available during the next decade, many pigs

will assume a major role in research laboratories. In fact, an appreciable increase in usage is predicted before this lower weight is realized.

Increased use of pigs and miniature pigs in the areas of genetics, nutrition, gastrointestinal and renal physiology, immunology, toxicology, radiobiology, and fundamental and clinical cardiovascular physiology seems assured. Additional information is needed to provide a firm basis for a broader assessment of the future role of pigs in biomedical research.

NATURAL SELECTION AND TRANSMISSIBLE DISEASE

AN informal meeting on "Natural Selection and Transmissible Disease", arranged on behalf of the Human Adaptability Sub-Committee of the International Biological Programme (IBP), was held at the Ciba Foundation on July 21–22, 1965. In his introduction, Prof. J. S. Weiner said that there were now some thirty national IBP committees and it was envisaged that the programme would include both world-wide surveys on blood groups and other genetical traits and also more intensive multidisciplinary studies on selected populations. Genetical constitution in relation to disease had been adopted as an IBP topic, but so far there had been no discussion on the prospects of research or the methods to be used.

Speaking on "The Genetical Analysis of Disease Susceptibility", Prof. L. S. Penrose gave examples of inherited disease resistance in plants and animals, and pointed out that genetical analysis of this phenomenon in man was much more difficult. It was particularly difficult to disentangle the effects of inborn and acquired immunity and this made the interpretation of familial concentrations of such diseases as tuberculosis and acute rheumatism uncertain. Traditional beliefs about the differing disease susceptibility of racial groups might often be mistaken, and attempts to correlate body-build with disease susceptibility had not been convincing. Haldane initiated the modern approach in 1932, when he suggested that thalassaemia might protect against malaria, and Allison had provided evidence for this in the case of sickle-cell trait. In African peoples with high sickling rates, eradication of malaria could lead to a detectable fall of sickling frequency in three or four generations. Theoretical treatment of gene frequency dynamics was more complex for sex-linked genes and where several interacting alleles exist in the same population. He suggested that the effects of chromosomal abnormalities induced by viruses on susceptibility to other diseases might be worth investigating.

In the discussion, Prof. Penrose stressed the advantages of examining single-gene traits and questioned the value of twin-studies. Prof. Vogel thought that multifactorial characters should not be ignored and that useful data could be obtained from twins examined throughout their life-span. Dr. Kirk, Dr. Allison and Prof. Penrose discussed the interpretation of gene frequency differences between West Africans and American Negroes. Dr. Roberts suggested that genetical data might be collected on volunteers experimentally infected with the common cold. The relationship between susceptibility to disease and clinical severity was discussed by Sir Graham Wilson, Prof. Stuart-Harris, Dr. Omerod and Prof. Vogel.

Prof. C. H. Stuart-Harris spoke on the epidemiology of virus diseases. He said that their epidemiological behaviour was similar to that of bacterial diseases. Some, like poliomyelitis, were confined to man; others, like rabies, affected man by chance and did not spread; while others, which are spread by insect vectors, are maintained in the population by a cycle of transmission. Some virus diseases have been established for many centuries, and epidemics of these may be due to changes in virus virulence, host susceptibility or conditions influencing transmission. Viruses are well known to be plastic in the laboratory, but as yet it has not been proved that virulence changes underlie major epidemics. Viruses live in very close association with cells, and genetical variations in the host tissues would be expected to be important in susceptibility. Mouse strains differing in resistance to certain viruses such as yellow-fever had been bred and interspecific differences in susceptibility are well known. He gave examples of the varying relationship between attack-rates and severity of disease and the age of the subjects. He then discussed smallpox, influenza, yellow-fever and typhus. India and Pakistan are now hyperendemic areas for smallpox, and mortalities might reach 40–50 per cent in some epidemics. Yellow-fever is endemic in West and Central Africa and heavy mortalities have occurred in recent epidemics in the Sudan and Abyssinia. Typhus, which is now endemic in the Middle East, North Africa, China and other regions, formerly caused heavy mortalities especially in persons more than fifty years of age. The influenza virus is very plastic and epidemics tend to occur when a new strain emerges. The work of McDonald and Zuckerman had shown that blood group O was more frequent among persons who had been infected with influenza A_2 virus, and unpublished work confirmed this. He thought that there was still scope for genetical investigations on populations to which certain viruses had not yet spread.

In the discussion, Prof. Barnicot asked for examples of non-immune populations which might be favourable for genetical investigations, and Prof. Stuart-Harris mentioned the measles epidemic in Greenland and polio epidemics among Eskimos. He said that Type I polio virus was absent in Tristan da Cunha before the exodus. In reply to Dr. Harrison, he said that little could be deduced about individual variations in susceptibility by measuring antibody titres. Dr. Roberts mentioned his own work on ABO blood groups in relation to *Rickettsia* infections in Nigeria, and Dr. Spicer spoke of his analysis of periodicity in deaths from respiratory diseases.

In his paper on "ABO Blood Groups and Infectious Diseases", Prof. F. Vogel said that in much of the earlier work on this topic the samples were too small, but relatively high B and AB frequencies had been found among patients with tertiary syphilis. His own work on ABO groups and smallpox had been stimulated by geographical correlations and by the claim, which was in dispute, that vaccinia virus contains A-like antigen. It was best, he thought, to seek correlations between blood group and clinical severity, and in India his team had found that scarring was more severe in subjects of groups A, B and AB or of groups A and AB than in those of group O. Clinically more severe cases among fresh smallpox cases in Pakistan were more frequent in groups A, B and also AB compared with O. Groups A and AB were in excess in a sample of post-vaccination encephalitis cases examined in Germany. Work in Austria suggested that infantile diarrhoea was more severe in group A children than in those of group O; German data for 1956 and 1960–63, which he himself had analysed, showed the same trend, but it was reversed in data for 1957–59. He felt that these various lines of work were sufficiently encouraging to deserve further attention.

In the discussion, Dr. Garlick reported a small series collected in a smallpox epidemic in Nigeria; no relation was found between ABO group and either the incidence or severity of disease. Dr. Allison expressed scepticism about the effect of ABO groups on smallpox infections. The possibility of collecting more data on tertiary syphilis, post-vaccination encephalitis and infantile diarrhoea was

discussed. Sir Graham Wilson pointed out the difficulties of diagnosis of post-vaccination encephalitis.

In a paper on the epidemiology of protozoal diseases, Mr. W. E. Ormerod said that disequilibrium between host and parasite could be adjusted by either of them becoming adapted by selection; owing to short generation times, adaptation of the parasite was likely to be more rapid. Some protozoa such as *Entamoeba coli* cause no disability in man; others, like *Plasmodium vivax*, cause illness; and some, such as *Trypanosoma rhodesiense*, are generally lethal. A benign parasite may be more harmful if other parasites are present. West Africans and American Negroes seem to have a solid immunity against *P. vivax* which is presumably genetical. This parasite may have been an important selective agent before equilibrium with man was achieved. Marked differences between certain cattle breeds in resistance to trypanosomiasis are known. This disease is enzootic in cattle but sporadic in man, and probably exerts little selective pressure on the latter. *Toxoplasma* is endemic in some human communities and may be a significant cause of abortion and still-birth. In general, if parasites can change rapidly, as can trypanosomes in contrast to plasmodia, host adaptation cannot proceed far in any direction. The highest selective pressures are likely to occur where a disease is holoendemic.

The epidemiology of toxoplasmosis and the immunity of Negroes to *P. vivax* were discussed. Dr. Allison considered that the latter phenomenon was genetical but not associated with haemoglobin variants or with G-6PD (glucose-6-phosphate dehydrogenase) deficiency.

Speaking on the epidemiology of bacterial diseases, Sir Graham Wilson said that the proportion of clinical, subclinical and latent infections and of uninfected people, both susceptible and immune, would depend on many factors. 98 per cent of people might catch a disease such as measles if it were introduced into a virgin population, whereas infectious anaemia of rats was widespread but latent. Probably most people are susceptible to plague and cholera, but these diseases do not occur to-day in Britain because conditions for transmission are unfavourable. Very little is known about genetical factors in disease resistance. Wide differences in the incidence of tuberculosis in troops of various nationalities in the First World War had been noted and in underdeveloped areas the disease assumes a rapidly progressive form when first introduced, but it is uncertain to what extent genetical differences are involved. Concordance work on twins is usually considered to show that genetical factors influence susceptibility to tuberculosis, but in a recent careful investigation Simonds attributed differences in twin responses mainly to environment. Certain cattle breeds and also mouse strains are known to differ widely in resistance to tuberculosis; in resistant mice there is better mobilization of mononuclear phagocytes, and strains resistant to *Salmonella typhimurium* have higher lymphocyte counts. Sir Graham Wilson thought that geneticists should direct the attention of epidemiologists to various simply inherited traits in the hope that they might be able to suggest possible connexions with disease resistance.

The role of nutrition in resistance to infections was discussed. Dr. Spicer said that both genetical and environmental factors were doubtless involved in susceptibility to tuberculosis. There had perhaps been a large epidemic of this disease reaching a peak in England in the nineteenth century. The interpretation of work on twins was further discussed and also the reasons why cholera tends to remain endemic in Bengal though occasionally breaking out elsewhere. Dr. Roberts had compared local variability in disease incidence in Nigeria with variability in blood groups and failed to find suggestive trends.

Dr. A. C. Allison spoke on "Genetical Components in Disease Resistance". He gave examples of single gene factors in bacteria conferring phage-resistance and in phages influencing infectivity. Resistance of insects to insecticides had provided striking examples of evolutionary processes. In higher animals the picture is complicated by various immune responses. Evidence from work on bacteria, phage and on some mammals and birds indicated that genetical changes in cell surface components might affect susceptibility to attack by parasites. The susceptibility of certain mouse strains to polyoma and mouse hepatitis virus is greatly increased by thymectomy at birth. The Aleutian mink gene affects coat colour and also leucocyte morphology; the homozygotes are susceptible to a 'collagen disease' induced by a filterable organism. Dr. Allison summarized the work on sickle-cell trait and malaria and considered that the evidence for a protective effect was now quite impressive. Both in Central America and the Caribbean sickling rates tend to be low where selection due to malaria has been relaxed. Sickling rates in various regions correlate well with rates of G-6PD deficiency. He pointed out that ABO blood group heterozygotes such as AO and BO would presumably be handicapped in producing antibodies to two types of antigen and might therefore be at a selective disadvantage.

In the discussion, Prof. Vogel stressed that stable equilibrium due to heterosis might not be important in the case of ABO blood groups; one disease might push the frequency of a gene up and another push it down. Mechanisms other than heterosis for achieving equilibrium were mentioned by Prof. Penrose and Dr. Harrison. Experimental work on malarial infections in relation to sickling and G-6PD deficiency was discussed by Dr. Kirk and Dr. Allison. Dr. Edwards doubted whether protection against malaria by sickling should be regarded as an example of heterosis and whether this was the important mechanism in the case of blood group polymorphisms. Dr. Roberts mentioned his own work on both G-6PD deficiency and sickling in Nigeria.

Summarizing and commenting on the meeting, Prof. Barnicot said that many examples of disease resistance due to single genes in animals and plants had been cited, but the detailed mechanisms of gene action were generally not clear. Work on animals might suggest fruitful lines of research, but it showed only what might happen in man and not what does happen. On the whole, the meeting had emphasized the pitfalls more than the possibilities of work on genetical factors in disease resistance.

Lines of attack on this problem include (*a*) twin studies; (*b*) the study of disease incidence in families; (*c*) the genetical comparison of affected and control samples; (*d*) correlation of the geographical distributions of genes and diseases. Twin data are difficult to collect, and analysis of disease susceptibility cannot be pressed very far. Family studies are complicated by acquired immunity, and heterogeneity for age of subjects and infective dose. Comparison of affected and control populations may require very large samples to show convincing effects, though small selective pressures may be important in evolution. It may also be difficult to obtain a valid control sample. Many genes and many pathogens are known, and a blind search for associations is likely to be both fatiguing and futile. The examination of single gene traits clarifies the genetical aspect of a complex situation; in the case of multifactorial characters we may not know how much of the phenotypic variation is environmentally induced and disease resistance may depend on hidden effects of contributory genes rather than the visible phenotype. Geographical correlations may have been disturbed by relatively recent population movements and historical data on these may be meagre. In studying immigrant populations there are uncertainties about the exact origins of the migrants and the extent of intermixture in their new home. Genetical investigations of epidemics need special organization if good material is to be collected, since local conditions may not be favourable for scientific work.

N. A. Barnicot

A SIMPLE ASSESSMENT OF PARTITION DATA FOR CORRELATING STRUCTURE AND BIOLOGICAL ACTIVITY USING THIN-LAYER CHROMATOGRAPHY

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SINCE the work of Overton[1] and Meyer[2] the conditions for the penetration of compounds into cells and the structure of cell membranes have been the subject of continuous investigation. Collander[3] has shown that the rate of migration of non-electrolytes into and out of the *Nitella* cell can be closely correlated with their partition coefficients between water and a number of organic solvents. Other factors such as molecular dimensions, hydration, adsorption and hydrogen bonding undoubtedly influence the rate of penetration, but the correlations observed for a number of biologically active compounds lead one to believe that partition coefficient is of overriding importance in controlling penetration of non-electrolytes into living cells[4].

Hansch and Fujita[5], and Fujita, Iwasa and Hansch[6], have recently proposed a general mathematical relationship between the penetration of a molecule into cells and a substitution constant, π, which they support with excellent structure/activity correlations obtained with eight different classes of compounds, each assayed against a specific test organism. The constant π is based on partition coefficient and is defined by the free-energy relationship:

$$\pi = \log (\alpha_X/\alpha_H)$$

α_H = partition coefficient of a parent compound
α_X = partition coefficient of a derivative

The direct determination of partition coefficient is tedious and often presents practical difficulties, particularly when the compound is highly insoluble in either of the solvent phases. We have therefore turned to a simplified assessment of partition coefficient and its derivatives by exploiting the theoretical relationship between partition coefficient (α) and R_F value deduced by Martin[7] for liquid–liquid partition chromatography:

$$\alpha = K\,(1/R_F - 1), \text{ where } K = \text{constant for the system.}$$

Bate-Smith and Westall[8] introduced the term R_M, where $R_M = \log (1/R_F - 1)$. The change in the value of R_M for a substituent (ΔR_M) is a free energy-based constant identical with π used by Hansch. It is therefore possible in principle to correlate the penetration of substances with their R_M values. Preliminary results were encouraging, and we therefore investigated the system further and found that for this purpose the chromatographic method has many advantages over the conventional method of measuring partition coefficient.

We chose a series of *N*-*n*-alkyltritylamines for investigation (Table 1) because their observed molluscicidal activity can be attributed primarily to different rates of penetration to the site of action. An analysis of the toxicity of a range of trityl compounds[9] has indicated that activity is associated with nucleophilic attack at the quaternary carbon atom of the trityl group:

$$Ph_3C{-}NH{-}R \xrightarrow{H^{\oplus}} Ph_3C{-}\overset{\curvearrowright}{N}H_2{-}R \rightarrow Ph_3C{-}X + NH_2{-}R$$
$$\uparrow X^{\ominus} \quad \oplus$$

Molluscicidal activity depends on the rate of penetration to the site of action and the facility with which the leaving group can be displaced when at that site. In the homologous series of *N*-*n*-alkyltritylamines the same toxicant is formed from all compounds and the relatively small differences in the nature of the leaving group have been found to make a negligible contribution to the observed activity. This can therefore be ascribed to different rates of penetration. The compounds were prepared by heating a solution of triphenylchloromethane (0·01 mole) in dry acetone (10 ml.) with the appropriate *n*-alkylamine (0·02 mole) for 1 h under reflux. The mixture was poured into water and the solid product was collected and recrystallized several times from ethanol, or, in the case of the hexyl-, heptyl- and octyl-derivatives, which were liquids, the compounds were isolated from the aqueous mixture by extraction with ether and were purified by chromatography on neutral alumina with hexane as eluent. Melting points and other data are given in Table 1.

Table 1. THE R_M VALUES AND MOLLUSCICIDAL ACTIVITIES OF *N*-*n*-ALKYLTRITYLAMINES

Compound $Ph_3C.NH.R$ $R=$	Melting point ° C Found*	Literature	LD_{50} estimates and 95 per cent fiducial limits (mg/l.)		Mean R_M value	Inter-plate standard error
Methyl	71–2	73†	2·10	1·94 –2·31	−0·305	0·045
Ethyl	74–6	75–7†	0·138	0·113–0·165	−0·119	0·034
Propyl	69–71	70–2†	0·046	0·043–0·049	0·008	0·036
Butyl	51–2	52·5–53·5‡	0·035	0·033–0·037	0·115	0·031
Pentyl	42§	—	0·054	0·049–0·061	0·225	0·018
Hexyl	Oil§	—	0·633	0·521–0·794	0·368	0·038
Heptyl	Oil§	—	31·65	25·67 –39·65	0·485	0·029
Octyl	Oil§	—	> 50	—	0·620	0·030

* Uncorrected.
† Beilstein's *Handbuch der Organischen Chemie*, **12**, 1344.
‡ Iliceto, A., Fava, A., and Mazzuccato, U., *J. Org. Chem.*, **25**, 1445 (1960).
§ Novel compound for which satisfactory analytical data were obtai ned.

Chromatography was carried out on glass plates (20 × 20 cm) coated with a 250μ thick layer of 'Silica Gel *G*' (Merck). After drying at room temperature for 2 h and then at 105° for 10 min the plates were impregnated by allowing a 5 per cent solution of liquid paraffin BP in hexane (v/v) to run to the top of the plate; the solvent was then evaporated at 40°. The compounds (3 μg/spot) were applied at 1·5 cm intervals along a line 2 cm from one edge of the plate so as to be 1 cm above the level of the developing solvent. In order to avoid any systematic error, compounds were applied to positions from a set of pre-determined random allocations, none being nearer than 2 cm to either margin. In addition, *N*-tritylmorpholine was run on all plates as a standard. The plates were developed by the ascending technique in a chromatography tank under conditions of equilibrium. The mobile phase, aqueous acetone saturated with stationary phase, was allowed to run for approximately 100 min when the solvent front had advanced 14 cm from the origin. The plates were then dried and the *N*-*n*-alkyltritylamines

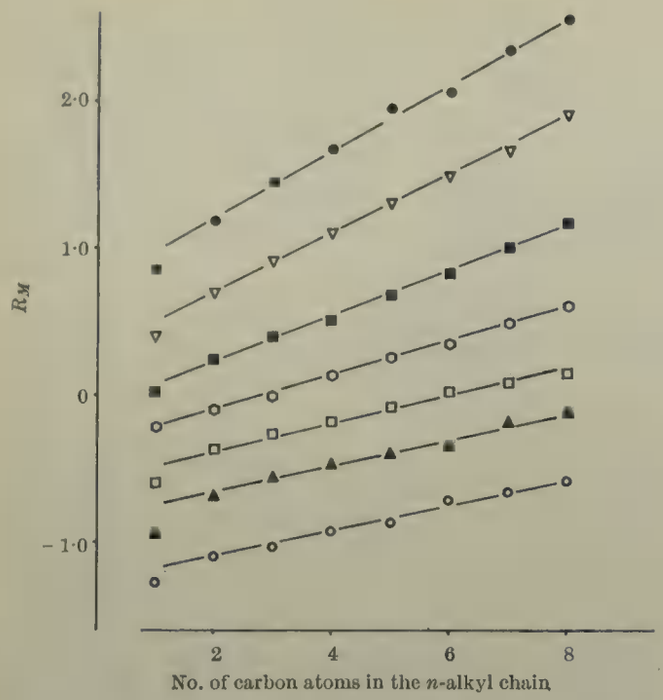

Fig. 1. Relationship between R_M values and number of methylene carbon atoms in the *n*-alkyl chain of *N*-*n*-alkyltritylamines in a series of acetone/water mixtures. With the exception of the Cl compound there is a linear relationship in all systems. Proportion of acetone (v/v) in the mobile phases: ■, 0·50; ▽, 0·56; ■, 0·67; ⬡, 0·75; □, 0·82; ▲, 0·83; ○, 0·91

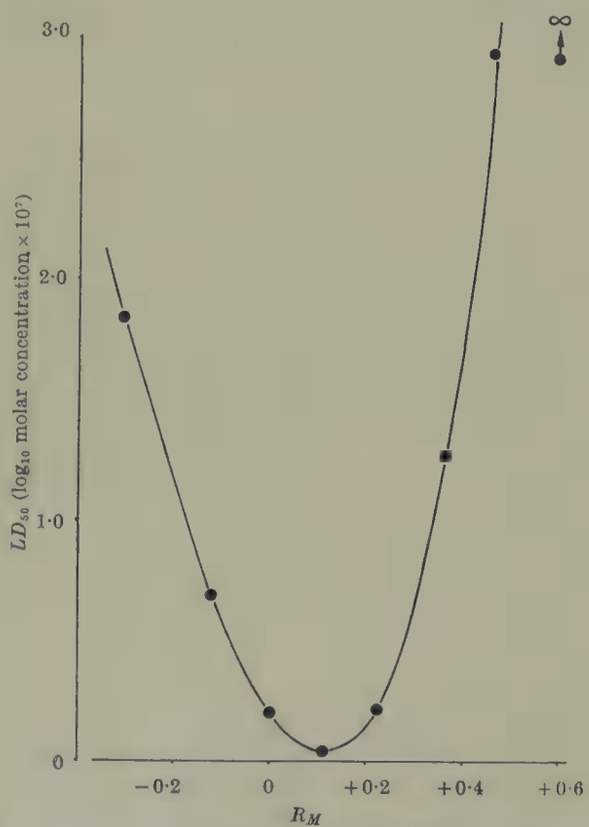

Fig. 2. Relationship between LD_{50} ($\log_{10}$ molar concentration × 10^7) and R_M when the proportion of acetone (v/v) in the mobile phase was 0·70

were detected by spraying with a solution of trichloroacetic acid (5 per cent w/v) in acetone. Yellow spots of the triphenylmethyl carbonium ion were produced after heating the plates at 100° for 5 min and, although these faded during the next 2–3 h as moisture was absorbed, they could be restored by repeating the detection procedure.

Estimates of LD_{50} (Table 1) were computed from dose-mortality data obtained with the snail *Australorbis glabratus*. Details of the procedure will be reported elsewhere[9].

Since these compounds obey the Martin equation, that is, there is a linear relationship between R_M and the number of methylene carbon atoms in the side-chain (Fig. 1), we have related the biological activity of these compounds with their respective R_M values (Fig. 2). The quadratic relationship observed has been found in other chemical series and is attributed by Hansch and Fujita[5] to an increase and decrease in the rate of penetration as the R_M values in a series change progressively and pass through an optimum.

The choice of chromatographic method is a matter of convenience; we have found no important differences between paper and thin-layer chromatography except that the latter is quicker and marginally more reproducible in our hands. Water is a constituent of the cell membrane and the protoplasm, so its use in one phase of the model system is obvious, but the lipoidal constitution of the cell membrane is unknown and the choice of hydrophobic phase must be somewhat arbitrary. From thermodynamic considerations, the nature of the non-aqueous phase should not affect the results qualitatively[10] provided that there is no hydrogen bonding. Collander's results[3] support this in that ether/water and olive oil/water partition coefficients are equally correlated with penetration into *Nitella* cells.

Protein is absent from the aqueous and non-aqueous phases used for direct partition coefficient measurements. Therefore, the contribution of the proteinaceous material in a cell membrane is tacitly excluded from the model system. The justification for this is that the variation in penetration can be almost entirely accounted for by partition coefficient data. We were unable to detect any effect on the R_F values of the *N*-*n*-alkyltritylamines when casein was incorporated into the silica gel support of the chromatogram (1 : 5 w/w) and we have therefore omitted protein from the chromatographic system for assessment of R_M values. We chose the reversed-phase system liquid paraffin/aqueous acetone with 'Silica Gel *G*' as support, although other materials, for example, alumina or Whatman No. 4 paper, impregnated with liquid paraffin were equally satisfactory and the R_F values were similar with all three types of support.

Using 'Silica Gel *G*' impregnated with liquid paraffin and 7 : 3 acetone/water (v/v before mixing) as mobile phase, it was possible to obtain R_F values for all members of the series of tritylamines from methyl to octyl in the range of maximum accuracy (0·2–0·8) (ref. 11). However,

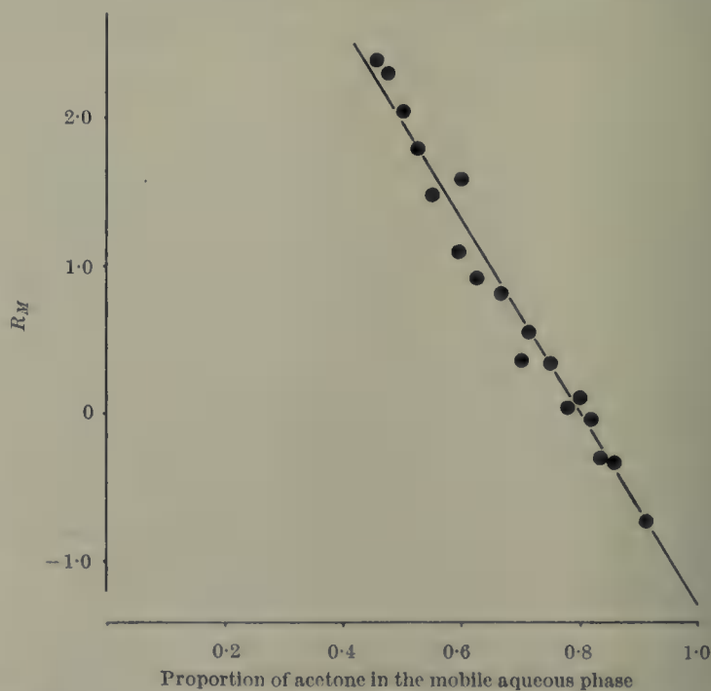

Fig. 3. The effect of solvent composition on the R_M of *N*-*n*-hexyltritylamine. Each point is the mean of at least ten determinations

in another class of biologically active compounds, the substituted benzonitriles[12], no single solvent system would give satisfactory R_F values for the most lipophilic and hydrophilic members which remained on, or ran close to, the origin and solvent front, respectively. We were able to overcome this difficulty and to extend the scope of the method by examining the R_F values of the tritylamines with mobile phases containing varying concentrations of acetone. Soczewinski and Wachtmeister[13] have shown a linear relationship between R_M and solvent composition, provided that the solvent mixtures did not deviate markedly from ideal solutions. When the composition of the mobile phase was adjusted systematically, this relationship was confirmed for the tritylamines (Fig. 3). Given preliminary chromatographic data with a variable proportion of water in the mobile phase, it is possible to choose a solvent mixture which will give not only R_F values in the range of maximum accuracy for most of the compounds, but also maximum R_M increments (Fig. 1). The R_F values which fall outside these limits can be measured in a number of suitable mixtures and the R_M values for the primary system can then be obtained by extrapolation.

The conditions necessary for obtaining accurate and reproducible R_M values have been described in detail by Bate Smith and Westall[8] for paper chromatography and have been reviewed by thin-layer chromatography, for example, by Stahl[14] and by Truter[15]. The mean R_M values for the tritylamines, together with their respective inter-plate standard errors, are given in Table 1. The greatest source of error is the inter-plate variation, which is 6·5 times greater than the intra-plate variation, which was assessed by replication of a standard on each plate. Since there was no evidence that the inter-plate variation was dependent on the R_M value, the estimate of error could be pooled to give a standard error or 0·033 for a single determination and the standard error of the mean R_M value from nine determinations was 0·011. This is 1·2 per cent of the total range of R_M values examined. The mean standard error of the estimates of the log LD_{50} values expressed as a percentage of the range of these estimates is 2·6 per cent. The accuracy with which R_M can be determined is therefore adequate for structure/activity correlations because of the difficulty of assessing biological response with equal precision.

The measurement of the R_M values by reversed-phase chromatography has a number of advantages over the conventional partition measurements, the most important feature being that it is much quicker and less tedious because fewer manipulations are involved. Up to twenty five compounds can be run simultaneously on one 40 cm × 20 cm thin-layer plate, so R_M values can be compared directly. The detection of spots by simple unspecific methods, for example, by iodine absorption or by the use of a fluorescent indicator, avoids the need for specific, quantitative analytical methods. As a corollary of this, it follows that impurities present will be detected or, alternatively, the material need not be pure provided that the salient spot can be identified. If necessary, the compounds can be extracted from thin-layer material

The measurement of partition coefficients of sparingly water-soluble compounds demands a long period of equilibration to ensure thorough partitioning between the phases and then colloidal material has to be removed by centrifugation. After this there still remains the difficulty of measuring low concentrations of the solute. Reversed-phase paper or thin-layer chromatography in a range of solvent mixtures can give R_M values for any of these mixtures by extrapolation provided that the linear relationship between R_M and solvent composition is established. Very little material, often less than 1 μg, is required for detection. When the layer is overloaded the spots streak and such plates should be discarded, thus ensuring that the results will not be affected by the presence of colloidal material or by the degree of saturation of the phases by the substance under investigation. The chromatographic method provides a rapid reproducible means of obtaining quantitative partition data for many types of compounds that can be used to assess the roles of penetration and inherent effectiveness in the expression of biological activity. With the growing use of multiple regression techniques for the analysis of this kind of problem the importance of such data is likely to increase.

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SPECTRAL DENSITY FUNCTIONS OF HYDROMAGNETIC EMISSIONS AT HIGH LATITUDE

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IN this article the regular emissions in the frequency range 0·5–5 c/s are considered. Interest in them has increased considerably during the past five years, but the approach to the problem adopted here is somewhat different from that employed hitherto by other workers. The nomenclature for this type of oscillation is rather complex. In 1964 the IAGA committee (cf. Jacobs *et al.*[1]) recommended that the term Pc 1 should be used for regular oscillations between 0·2 and 5 c/s, but many workers have also referred to these signals as hydromagnetic emissions, pearl pulsations, 'type A' oscillations, etc. (compare, for example, Troitskaya[2], Campbell[3], Gendrin and Stefant[4], and Tepley[5]). In what follows the term 'hydromagnetic emission' (HM) will be used to describe the regular oscillations in this ultra-low-frequency range.

The principal detection systems used normally comprise iron-core loops (or air-core), low-noise amplifiers (between 0·1 and say, 10 c/s), chart and/or magnetic tape records. The use of tape makes possible careful analysis after the phenomenon has been observed. The HM emission data obtained have normally been presented in one or both of the following two types of displays: (*a*) amplitude against time, and (*b*) frequency against time (sonagrams). Both these methods of presenting data offer much valuable information concerning the micropulsations.

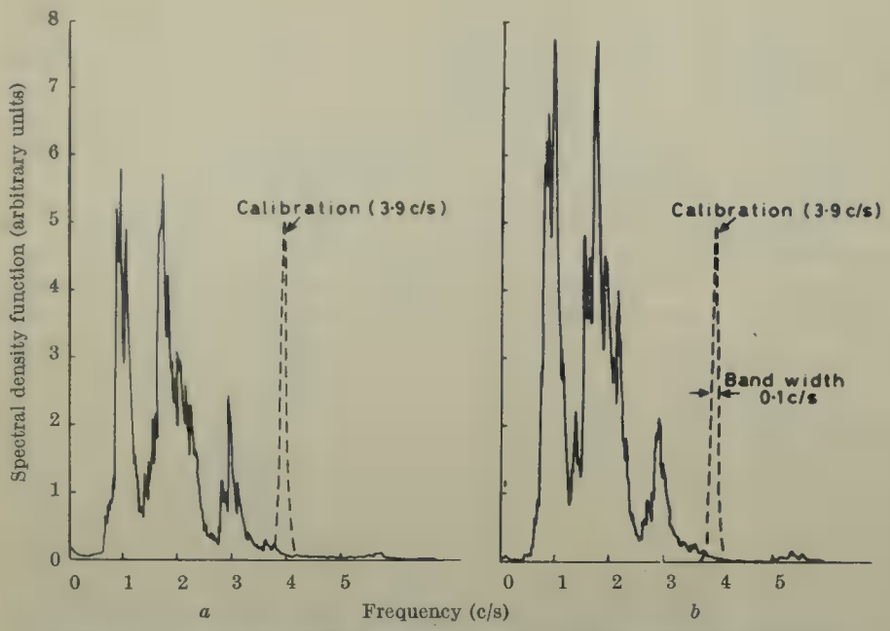

Fig. 1. Spectral density function for the Tromsö hydromagnetic emission record on December 9, 1964. *a*, Record between 2147 and 2149 M.E.T..; *b*, record between 2151 and 2153 M.E.T.

However, if we take the resolution time on the sonagrams this is normally no better than the order of 10–30 sec, and the signal amplitude of sonagrams can only be read off with an accuracy of about 20–30 per cent. This means that the bandwidths as well as the mid-band frequency of ultra-low frequency emissions cannot be determined accurately. Concerning the amplitude against time display, Pope[6] has shown that these records must be used very cautiously when more than one emission frequency occurs, as the amplitude is strongly dependent on both the number and amplitude of beating frequencies in the emissions.

I therefore feel that if one could find a simple and accurate method of evaluating spectral density functions of HM emissions, it would be of great additional value for the two types of data presentation mentioned here; especially where bandwidths, centre-frequency and power density are concerned. Power spectra can, of course, be evaluated from amplitude-time displays, but the normal methods for reduction and computation of power spectra are time-consuming and not favourable for statistical analysis (cf. Vozoff *et al.*[7]).

This communication describes an accurate and rather simple method for power spectral analysis of micropulsations, and demonstrates two examples of HM emissions recorded in this way. The measurements were started at the Auroral Observatory at Tromsö ($\Phi = 70°$ N., $\lambda = 19°$ E.) last autumn. The rapid geomagnetic fluctuations were received on an air-core loop antenna, with an effective absorbing area of approximately 3,000 m² and with the axis located in the magnetic N.–S. direction. A d.c. preamplifier, with maximum gain of approximately 100 dB for 1 μV input, was used. With a frequency-modulated adapter (cf. Mohus[8]) connected to a magnetic tape-recorder, it is possible to record (with a tape speed of 7·5 in./sec) signals between d.c. and approximately 200 c/s. All the instruments were transistorized and battery-powered.

The tape-records are analysed on 'ISAC', a statistical analogue computer, designed and built at the Automatic Control Laboratory of the Norwegian Institute of Technology. ISAC computes correlation functions, power spectra and first-order distribution functions for signals in the frequency range 0–200 c/s. All computations, which are performed at high speed, are done automatically and the results are plotted direct by an *xy*-pen recorder. The resolution in frequency and power is high (see below). Frequency- and amplitude-calibrations are automatically carried out. ISAC weighs approximately 40 kg and may easily be carried around. (For further details concerning ISAC, compare, for example, Balchen and Blandhol[9].)

Two samples of spectral density function of HM emissions evaluated by this method shown in Fig. 1*a* and *b*. The calculation and plotting of each power spectrum take about 8 min. No attempts at smoothing the curve have been made. Fig. 1*a* displays the spectrum recorded on December 9, 1964, between 2147 and 2149 M.E.T., while Fig. 1*b* illustrates the spectral density of HM emission recorded 2 min later the same night: also in this case only 2 min of actual recording on the tape has been used in the analyses. The frequency accuracy is better than 0·1 c/s for both spectra.

As easily seen from the curves in Fig. 1, three dominant narrow bands are observed. Mid-band frequency as well as the bandwidth of the HM emissions can be measured very accurately direct from the curves. The centre frequency of these bands in Fig. 1*a* is 0·95, 1·7 and 2·95 c/s, while the corresponding figures for Fig. 1*b* differ by less than 0·2 c/s. The frequency widths (half-widths) for these three pronounced bands shown are 0·18, 0·32 and 0·1 c/s measured from the origin, while the corresponding bandwidths in Fig. 1*b* are 0·4, 0·13 and 0·2 c/s. The ratio between the band-width and the centre frequency ($\Delta f/f$) varies from 0·4 to about 0·03; thus the pulsations of HM emissions are normally confined to a very narrow frequency-band.

On first viewing these emission curves, it may look as if the second and third peak (measured from the origin) are the second and third harmonics of the first band, but the figures given here show that this is not the case. However, it can be objected that a rather large uncertainty exists in defining mean periods and bandwidths when such relatively short intervals of data are used. On the other hand, as these two curves are almost identical (except for the fine structure), both the mid-band frequency and the bandwidths of this HM emission should be significant.

It is difficult to devise accurate calibration for measurements of this type and, so far as I know, very little has been

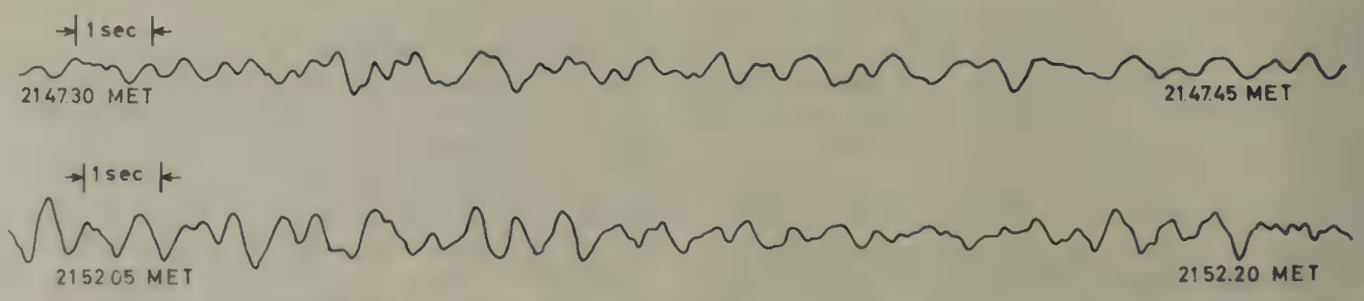

Fig. 2. Amplitude-time display. Record on December 9, 1964: upper, between 21.47.30 and 21.47.47 M.E.T.; lower, between 21.52.05 and 21.52.22 M.E.T.

written concerning signal strength of ultra-low-frequency emission. Unfortunately, no satisfactory calibration was carried out during December 9. But from later measurements it seems possible to conclude that the peaks in the spectra shown in Fig. 1 correspond to a variation in the magnetic field of the order of a few mγ $c/s^{-1/2}$. This implies that the ultra-low-frequency emissions contain rather low energy compared with micropulsations in the frequency range between, say, 0·1 and 0·001 c/s. But, on the other hand, the amplitude resolution with this type of data presentation (cf. Figs. 1*a* and *b*) is very high.

That fine structure exists (superimposed on the regular band emission) is seen in the spectral density functions. However, detailed studies of fine structure of HM emissions can be made more directly and simply from frequency-versus-time displays (sonagrams).

With the other methods of presenting data that have been mentioned, it is very difficult to determine, with extreme accuracy, bandwidths and mean periods. This is especially true for the amplitude-time display. To illustrate this better, a small part of the amplitude-versus-time records obtained during the same time interval as the magnetic tape data used for the plotting of two power spectra discussed here, are shown in Fig. 2. The wave-form display varies more or less symmetrically about a base line but, as is easily seen, the modulation pattern is rather irregular, and they scarcely seem to exhibit a characteristic modulation frequency. Therefore, an interpretation of the spectrum based, for example, on the peak-to-peak interval can be completely misleading. It is also impossible to arrive at the number of emission bands directly from such wave-form display.

Furthermore, it should be pointed out that the two spectral density functions of HM emissions, described here, were recorded during magnetically quiet conditions and no riometer absorption occurred during this time. Also, during the 12 h immediately before and after this time of observation, scarcely any magnetic- and/or riometer-deflexions could be seen on the diagrams.

Many other power spectra, with quite different shapes, have been obtained. These results together with theoretical considerations will be discussed in a later report.

This work was supported by the Cambridge Research Laboratories, OAR, through the European Office, Aerospace Research, U.S. Air Force, contract *AF* 61 (052)–811. I thank Mr. I. Mohus of the Norwegian Institute of Technology for help with the power spectra analysis.

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COINCIDENCE OF MAGNETIC DISTURBANCES WITH LOCAL EARTHQUAKES RECORDED FROM THE ETHIOPIAN RIFT SYSTEM

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LABORATORY experiments[1–7] have been carried out to investigate the behaviour of rock magnetic susceptibility under conditions thought to approach those at focal depth prior to, or at the moment of, an earthquake: slow stress accumulation, sudden shear stress release, various Earth magnetic field intensities and orientations using different rock types, etc., in order to foresee what kind of magnetic anomalies might possibly be expected in the local magnetic field when an earthquake occurs.

Some authors have even expressed the opinion that some magnetic anomalies prior to a seismic event could even be used as a method for predicting earthquakes[7,9].

In general, however, authors do not agree[4,6,7] on the total validity of the laboratory experiments on the grounds that they either do not reproduce the exact conditions prevailing at the seismic focal points, or simply because earthquake mechanism is not yet completely understood[7] and varies according to local geological or tectonic conditions. I presume that the study of well-controlled field data may provide a more solid basis to the discussion of the problem. What, in fact, do geophysical stations equipped with both magnetic and seismic recorders register prior to or during a seismic event of a given magnitude (M) occurring at limited epicentral distance?

The Geophysical Observatory at Addis Ababa (09° 02′ N., 38° 46′ E.) is in a favourable position to answer such a question. About 175–200 km north-north-east from the station, on the western escarpment of Afar, lies the very seismically active region of Kara-Kore, from where more than 3,000 shocks were registered during the summer of 1961; two of these shocks were of $M \geqslant 6{\cdot}2$. At a similar distance to the south-south-west on the floor of the Main Ethiopian Rift is the dormant volcano Chubbi; this region is less active than Kara-Kore, but nevertheless experienced in 1960 an earthquake of $M = 6{\cdot}3$. Thirdly, 500–600 km to the north-east of Addis Ababa is situated the seismically active region of the Gulf of Tadjura.

Since its opening, the magnetic station at Addis Ababa has been equipped with three standard Ruska variographs (H, D and Z) operating at sensitivities of about 3 γ/mm, with a time base of 20 mm/h. Prior to November 1962, the S_0 for D was 10 γ/mm.

In February 1965, two high-sensitivity rapid-run Selzer Fluxgate H and D magnetometers (supplied through the courtesy of the Institut de Physique du Globe, Paris) were put into operation with galvanometer periods of 0·5 and 4·0 sec, and with overall sensitivities ranging from 0·02 to 0·002 γ/mm adjusted according to the level of sferics. These flux-gate magnetometers are insensitive to seismic noise and long-period magnetic variations.

In order to detect any magnetic effects related to local earthquakes, all the magnetograms since March 1959 (date of opening of the AAE seismic station) were attentively examined for unusual magnetic disturbances prior to, simultaneous with, or following a local earthquake of magnitude $M \geqslant 5{\cdot}0$ (Richter intensity scale). Since the Ruska H and D variometers are of the pendulum type, they are sensitive to earth tremors and, under the violence of local shocks of the aforementioned magnitude, the photographic traces disappear for periods of time often as long as 9–10 min. It should be noted, therefore, that the magnetic disturbances discussed here and indicated as coinciding with Earth tremors may have taken place either suddenly, or gradually during this 'black-out' period.

Table 1

Seismic data					Magnetic data			
No.	Date	Origin time L.M.T.	Epicentral distance	M	ΔH	ΔD (γ)	ΔZ	Observations
1	1958, May 25	02 : 54	635 km	5·5	0	–	–	
2	1958, May 25	05 : 54	635	5·0	*R*	*R*	*R*	
3	1960, July 14	21 : 40	240	6·3	*R*	0	–	
4	1960, Aug. 8	15 : 28	715	5·4	0	0	0	
5	1961, Mar. 11	11 : 41	545	5·9	−1·5	0	0	ΔH may be due to normal magnetic activity
6	1961, May 29	07 : 60	205	5·0	−5	0	–	$\Delta H = 4\gamma$ about 36 min ahead (?)
7	1961, May 29	13 : 52	150	5·5	−20	0	0	Small pip at 13 : 30
8	1961, May 29	22 : 24	200	5·0	0	0	0	*H*-trace at night-level
9	1961, May 29	22 : 26	210	5·0	?	?	?	Effect covered by previous shock
10	1961, May 30	16 : 11	220	?	−1	0	0	
11	1961, June 2	02 : 29	210	6·4	−16	0	0	*H*-trace above night-level due to a magnetic bay
12	1961, June 2	07 : 51	195	6·2	−4	0	0	
13	1961, June 2	08 : 22	200	?	−12	0	0	
14	1961, June 2	08 : 45	215	5·8	0	0	0	
15	1961, June 2	10 : 03	175	5·5	0	0	0	Magnetic activity: $k = 5$, $C_1 = 1·3$
16	1961, June 3	18 : 23	205	5·8	?	?	?	Too many shocks to detect any reliable ΔH
17	1961, June 14	23 : 32	210	5·7	0	0	–	$\Delta H = 2$–3γ 42 min ahead (?)
18	1962, Nov. 11	18 : 15	915	5·6	0	–	–	*H*-trace at night-level
19	1963, Oct. 5	17 : 58	530	5·3	0	0	–	*H*-trace at night-level
20	1964, July 3	22 : 19	230	5·0	0	0	–	*H*-trace at night-level

R, Record missing. –, Event not recorded on that component.

Table 1 sums up the observations on Ruska magnetograms for local earthquakes of $M \geqslant 5·0$ at the moment of the seismic registration. On these magnetograms, no abnormal magnetic disturbances have been detected either prior or subsequent to the Earth tremors. It must be emphasized that the ΔH, ΔD and ΔZ values indicated in Table 1 do not refer to the amplitude of the pendulums oscillations, but to a sudden permanent change in the intensity of the magnetic field. The station being situated at the magnetic equator, $H \approx F$.

From Table 1, it appears that at the moment of a local earthquake:

(1) No permanent change in declination (D) or in the intensity of the magnetic vertical component (Z) has yet been observed.

(2) Magnetic disturbances prior to the seismic events listed as Nos. 6, 7 and 17 could only questionably be related to these events and are in no way outstanding.

(3) Permanent changes in the intensity of the magnetic field (restoration of the field to its normal value) are observed at the time of the registration of local earthquakes and are always negative.

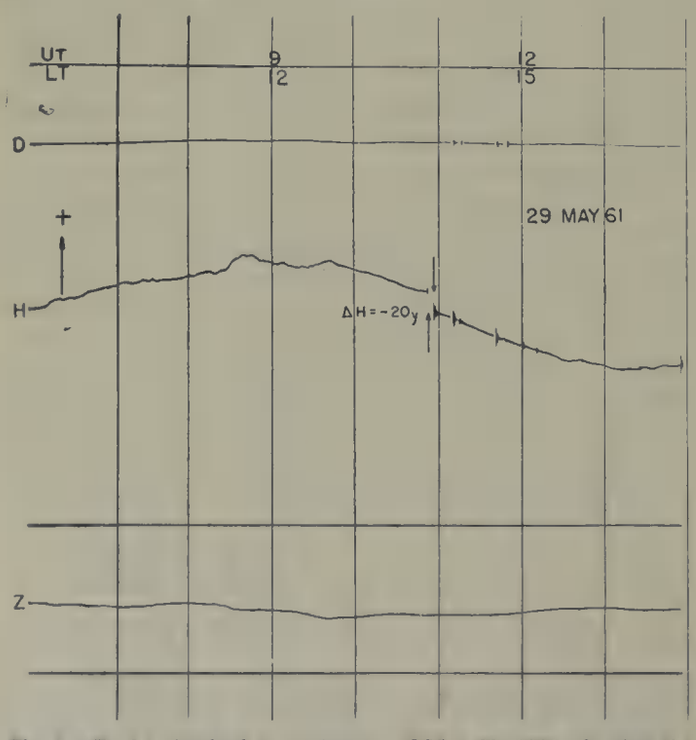

Fig. 1. Ruska standard magnetogram of May 29, 1961, showing the sudden decrease in the value of H at the moment of local earthquake No. 7 in Table 1. The tremors following that seismic event show no apparent change with intensity of the field

(4) Neglecting a possible effect of focal depth for tremors of approximately equal epicentral distances and magnitudes, the negative magnetic disturbances (ΔH) show a certain tendency to be enhanced during day-time, when the H (or F) trace is above night-time level (compare events Nos. 6 and 13 with Nos. 8, 17 and 20). There are, however, three apparent exceptions to this tendency.

(*a*) Earthquake No. 11 happened at night (L.M.T. 02 : 29) but shows a $\Delta H = -16\gamma$. The occurrence of this earthquake coincided with a magnetic bay when the H-trace was above the normal night-level. The decrease in intensity of the magnetic field by 16γ may either be due to the earthquake or may simply mark the end of the magnetic bay.

(*b*) Earthquakes Nos. 14 and 15 happened in the morning when the H-trace was well above night-level; the seismic magnitudes were respectively 5·8 and 5·5 and epicentral distances 215 and 175 km, but no magnetic disturbances were registered. Observations show, however, that this absence of magnetic disturbances prevails during a swarm of tremors. Earthquakes Nos. 14 and 15 were preceded by two shocks which released −4 and −12γ, respectively, within 56 min. Many other similar examples could be given. In a swarm of quakes, when tremors follow one another at short intervals, the total piezo-magnetic energy is released during the first shocks, and if a new build-up of piezo-magnetic energy does occur in such a short period of time (a few minutes) its intensity is too weak to be recorded 200 km away.

(5) The theoretical rate-of-change in the intensity of the Earth magnetic field during the hours or days preceding a major seismic event cannot be expected to be observed at magnetic stations under the influence of the equatorial electrojet; it would be hidden in the unpredictable variability of the daily *Sq* variation.

A question arises: Could the displacement of the H-trace on the Addis Ababa records, interpreted as a variation of the magnetic field at the moment of a local earthquake, be mistaken for seismically induced physical displacements of the working parts of the H-variometer? Such a possibility is very doubtful because:

(*a*) The variometers at the station are solidly cemented to their pier and their base can in no way be moved. Had they been moved under the seismic shock the base-line traces also would have been displaced, since the mirrors for these are attached to the base of the instruments.

(*b*) The mechanical effects would therefore be restricted to the pendulum, which is free to oscillate within the limits of its damping cup. One does not easily understand why, in a series of earthquakes of equivalent magnitudes and epicentral distances, a few shocks happening when

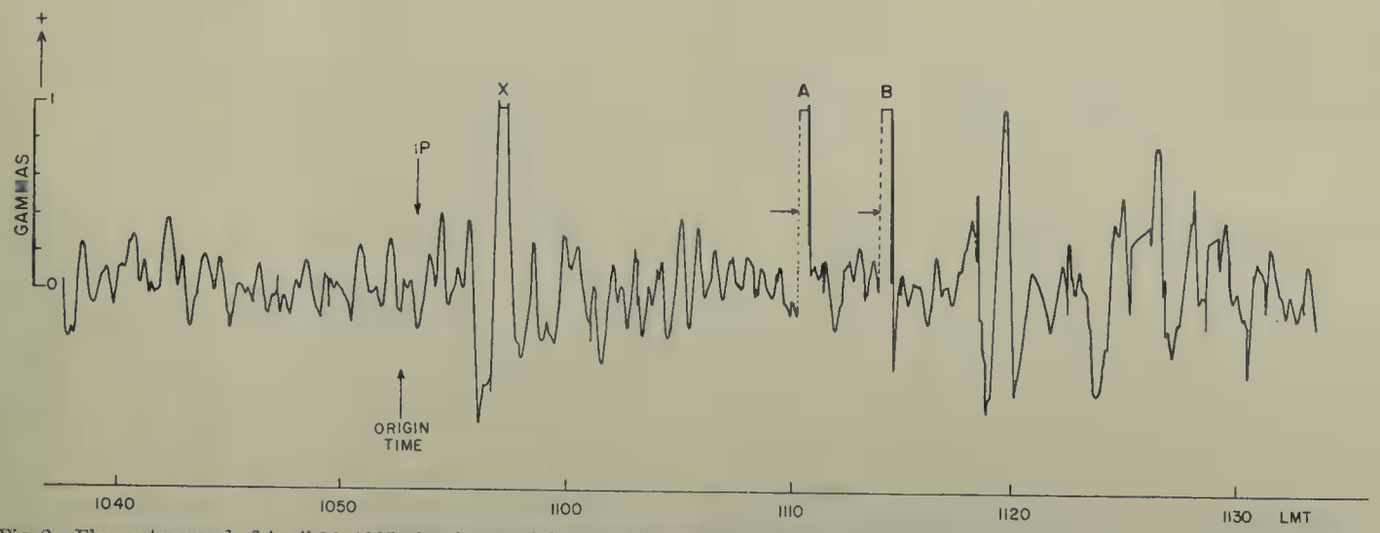

Fig. 2. Flux-gate record of April 14, 1965, showing possible magnetic effects of earthquake No. 6 in Table 2 (the record has been retraced by hand owing to the faintness of the trace)

the H-trace is above night-level would cause a physical disturbance on the H-pendulum when all the other shocks occurring at night-level hours did not produce any such physical disturbances (Table 1).

(*c*) The instruments have been submitted to a mechanical vibration test (because of the solidity of the pier, the seismic oscillations could not be exactly reproduced) and no permanent change in the position of the H-trace has been noticed. I think that seismic shocks as mentioned in (*b*) are still the best test.

Moore[9] reported an astonishingly sudden increase of 100γ in the intensity of the total field (F) recorded on a flux-gate magnetometer at Kodiak, 66 min before the Alaska earthquake of March 27, 1964. Kodiak was 440 km away from the epicentre. Unfortunately, Moore's comments are based on a single observation.

Breiner[10] mentions repeated events recorded just prior to local earthquakes, but although his records (*op. cit.* Fig. 2) show a positive disturbance 10 min before the seismic event studied, he refrains from commenting on this magnetic disturbance until more evidence becomes available.

The flux-gate magnetic records from Addis Ababa have been examined in correlation with local earthquakes, some of which are listed in Table 2. I admit that the choice of seismic events in Table 2 is not ideal, owing to generally low magnitudes and to epicentral distances higher than 300 km, but this cannot be remedied until more and bigger local earthquakes are experienced at Addis Ababa.

Table 2

No.	Date	iP (L.M.T.)	d (km)	M	Magnetic observations
1	1965, April 13	05 : 36 : 28	850	5·1	No magnetic disturbance
2	,, ,,	23 : 26 : 58	300	2·7	,,
3	1965, April 14	04 : 05 : 08	320	2·5	,, ,,
4	,, ,,	06 : 13 : 05	320	3·1	,, ,,
5	,, ,,	07 : 13 : 08	350	3·7	Small + pips at 06 : 55 : 35 and 07 : 17 : 10
6	,, ,,	10 : 53 : 11	350	4·3	See Fig. and comments below

Of the six events listed in Table 2, only the last one (d = 350 km, M = 4·3) showed any magnetic disturbances (Fig. 2) which could be termed unusual, but these certainly would not be differentiated from normal magnetic pulsations if it were not for the knowledge that a local earthquake had, in fact, occurred and that therefore seismo-magnetic effects might be expected.

Fig. 2 shows a part of the flux-gate magnetic H-trace recording for July 14. Both the origin time and iP-phase recorded time are indicated. The very small pips on the magnetic trace are lightning effects, well recorded on the D-trace. Prior to iP (10 : 53 : 11), no unusual magnetic disturbance could be detected. At 10 : 56, a large isolated sinusoidal pulsation (x) occurred, but this is nothing unusual on the flux-gate records. Later, at 11 : 11 and 11 : 14·5, two similar very sharp and quite unusual positive pulses (A and B) appeared at the end of 30-sec abrupt increases of the magnetic field. Note that these disturbances do not appear on the D-trace.

The last two negative disturbances marked A and B are presumed to be seismo-magnetic, but further observations are necessary before a cause-to-effect relation could be affirmed.

In conclusion, from the examination of the standard magnetograms at Addis Ababa, up to now at least, it appears that:

(*a*) No disturbance in the local magnetic field has been observed either prior to or following a local seismic event originating in the Ethiopian Rift system.

(*b*) Negative stepped decreases in the intensity of the total magnetic field ($H \approx F$ at Addis Ababa) have been observed coinciding in time with local Earth tremors. (Nagata and Kinoshita[6] would have expected $a + \Delta H$.) The amplitude of the decrease ΔF seems proportional to the magnitude of the earthquake (therefore to the stress accumulated), to its epicentral distance from the station, and most probably to the amplitude of the positive daily variation (S) of the magnetic field above the mean night level. Such a decrease in the intensity of F has also been observed by Breiner in Nevada[10].

(*c*) The seismo-magnetic effects produced no deviation in magnetic declination such as Miyakoshi[11] has observed in Japan.

Unexplained phenomena have also been noted on the high-sensitivity short-period flux-gate recording either before or following local seismic events. Their polarity seems to be positive while the permanent magnetic effects on standard magnetograms have been observed to be negative. However, the time of their appearance in relation to either the origin time of the earthquake, its iP recorded time at the station or its epicentral distance, is so inconsistent that much more evidence is needed before any conclusion could be drawn.

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RELATIVE SIMILARITIES IN ONE DIMENSION

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PERCEPTION, in man, is the act of deriving some meaning or message from signals transmitted to the brain from the sense organs. It consists of detection, in a set of simultaneous or sequential signals, of sets of interrelations among the signals, which we may call 'patterns'. The human brain, then, must have some mechanism for detecting patterns in signals and also for comparing recently received patterns with previously received ones. We do not know what this mechanism is, but when a pattern is recognized as a familiar one, we call the process identification. When a pattern is recognized as novel, the process is classification, or concept formation, because in the recognition of novelty we have added a new pattern to our repertory. In other words, we have added a new concept to our view of reality, or we have enlarged our perceptual sorting system for deriving meaning from signal input, or we have added a new class to our classification of all things.

This process begins at, or before, birth, and continues day by day and minute by minute. The result is a mapping, in the brain, of what we conceive of as reality. Reality for the individual can only be his map of what the world is like in relation to the signals he receives. 'Reality' in the sense of 'what the world is like in relation to man collectively' is the object of scientific investigation. We can say that whenever concepts about reality lead to reliable predictions, and hence useful responses, the concepts are valid ones. When they do not lead to reliable predictions or useful responses, the concepts are 'wrong'. Validity of concepts can only be judged on their utility, even though we may hope that the concepts of science form an undistorted mapping of the physical universe.

Of course, this process of concept formation, or development of pattern sorting systems, soon becomes complex. Without receiving new signals, we can detect patterns of patterns when we have a large store of them in our repertory (and perhaps even conceive of patterns not implicit in our repertory). This process we might call abstract conceptualization.

In the classification of bacteria, as in many problems in psychology, the attributes available for classification are largely non-visual. When the attributes in which we wish to perceive patterns are non-visual and not obviously convertible into visual form by any simple method. we are cut off from the superbly efficient, intuitive system which we have developed for sorting visual signal patterns. It is thus understandable that psychologists and bacteriologists have been leaders in attempts to develop numerical methods to substitute for visual ability in classifying. The attempts, so far, are somewhat unsatisfying, not only because they involve algorithms which may be different from our intuitive systems, but because they may yield different results. It therefore seems desirable to attempt to understand the intuitive mental procedures we follow to recognize and compare patterns, even though the mechanism by which we carry out the procedures remains unknown.

One of the many problems is to determine the procedure by which we judge one-dimensional similarities (that is, similarities among pairs of states of a single attribute). The method by which we distinguish qualitative similarity is obviously related to discriminatory ability. Things which we can discriminate between are 'different'; things which we cannot discriminate between are 'one' or 'the same'. This has been the basis of most numerical taxonomy so far. Single attributes are considered only as having qualitative similarity (0, 1 or +, −). In some instances similarities among single attributes have been scaled by considering them as sums of qualitative similarities (+ + +, + + −, + − −, − − −). Methods for weighting the sums have also been used[1]. However, so far as we can determine, no one has proposed a satisfactory definition for what is meant by quantitative or fractional 'similarity' in a single dimension[2].

The key to solving this problem lay in the use of a model. We assumed (hopefully without distortion) that states of any single attribute (values in a single conceptual dimension) were ordered and monotonically related to distance in a single Euclidian dimension. We then considered similarities among a number of lengths (distances in one dimension). Considering hypothetical, exact lengths, it became clear that quantitative similarity is not a function of discriminatory ability. The uncertainty in an estimate of similarity is related to discrimination, but the size of the estimate need not be. It was also obvious that quantitative similarity must be relative, since there is no similarity (other than 0 or 1) when one considers only two states in the absence of other states or a base line of some kind.

If you ask a person: "How similar are lengths of 1 and 2 units?" he may tell you they are 1/2 similar. This sounds as though similarity is simply the ratio of two lengths. Other people, given the same task, will say that the lengths have no similarity, or they will avoid answering in terms of similarity, indicating that they do not have any basis for judging this relationship. If you give a set of three lengths, $A = 1$, $B = 2$, and $C = 12$ units, and ask how similar are A and B, most people will say that they are quite similar, and will volunteer that A and B are more similar than A and C and further that B and C are more similar than A and C. There is no doubt in most minds about the rank order of the three pairs.

Similarity is obviously a ratio or proportion of some kind. But a proportion of what? We arrived at the answer to this question, appropriately enough, by gradually recognizing that we had backed past it some time before. As one of a number of attempts to code attributes in some way so that similarities could be weighted, we had tried the relation:

$$S_{AB} = \frac{L - |A - B|}{L} \qquad (1)$$

where S_{AB} is the similarity of states A and B, and L is the distance between the most dissimilar pair of states (C and D) in the set being considered. In our length model, the 'state of A' is the value of length A in the arbitrary scale units used.

Equation (1) specifies that similarities are fractional, that they range between 0 and 1, and that they are valid only within a given range of states. If the set of lengths is enlarged so as to increase the distance between the most dissimilar pair, the original fractional similarities must be shifted towards 1. Although the fractional values change, the rank order of the similarities does not (that is, the shift is monotonic). Thus, speaking of relative similarity is redundant, since all similarities are relative to some explicit or implied range. However, we would like to retain the expression 'relative similarities' as a

term for a 'set of similarities computed according to a single procedure for a specific set of things'.

Going back to the similarity between lengths of 1 and 2, we see that those who said they were half similar assumed that the lengths were a sample from a set ranging from 0 to 2, while those who said there was no similarity assumed that they were a sample of a set (or the whole set) ranging from 1 to 2. The inclusion of a third and longer length in the set increased the range and increased the similarity of lengths one unit apart.

Verbalizing equation (1) we see that 'similarity is the fraction of the considered range not included between two states'. We now accept this as the definition of similarity in one dimension, and suggest that if you substitute 'things' for 'states' in the foregoing definition, you have a good general definition for the intuitive concept of similarity in any number of dimensions. One reason that this concept has been so elusive is that the numerator is defined by stating what is not (the considered range not included). There was no word for this quantity and we propose that it be called 'undifference' (U). Undifference is the quantity which relates the distance that does exist between a pair of states to the distance that could exist between them. Or, stated another way, undifference is the distance that a pair of states is not apart. The intuitive idea that a pair of identical states drawn from a short set is not as undifferent as a pair of identical states drawn from a long set does not apply to hypothetical lengths, because we can distinguish an infinite number of such lengths in either a short or a long set. In the hypothetical case, the following relations hold:

$$\begin{aligned} L &= |C - D| \\ U_{AA} &= L - |A - A| = L = U \text{ for this system} \\ U_{BB} &= L - |B - B| = L \\ U_{AB} &= L - |A - B| \\ U_{CD} &= L - |C - D| = 0 \end{aligned} \qquad (2)$$

and hence equation (1) can be rewritten as:

$$S_{AB} = \frac{U_{AB}}{U}$$

Although the undifference is defined only to within an arbitrary, multiplicative constant (the scale used to define the states), the relative similarities are uniquely defined since the same scale is used for A, B, C and D. Undifference, as described here, was clearly anticipated by Reynolds[7] in his recent article on comparative elaborateness.

When we wish to use a concept, such as length, to indicate something about the nature of a real thing, uncertainty arises. We can stipulate, and hence know for certain, the state of a concept; but real things do not exist in our conceptual dimensions and states, they exist in reality (whatever that may be). Therefore, our only recourse is to stipulate tests or measures (physical sorting systems) which yield signals (measurements) which we will interpret as data (states) which we assume are applicable to our concept. A number of measurements, made by repeatedly applying the same measuring procedure, may be treated statistically to yield a mean value and a probability that the next measurement made by the same system would fall within a given distance of the mean. Unfortunately, statistics may not tell us whether our concept of what we are measuring fits what we actually are measuring, but this need not lead to error, so long as the deficiency is recognized. There are, then, two sources of uncertainty: (1) Lack of precision due to measuring error, (*a*) where imprecision is independent of the magnitude of the measurement over the range being measured, and (*b*) where imprecision is related to the magnitude over the range being measured. (2) Lack of precision due to inapplicability of the measuring system (non-unidimensionality in the quantity being measured, *vide infra*).

Let us assume first that the best estimate we can get for the state (value in arbitrary units) we are trying to determine is the mean or average of the measurements. It would seem reasonable to determine relative similarities according to these means. Thus, for equation (1) we could substitute:

$$S_{AB} = \frac{L - |\bar{A} - \bar{B}|}{L} \qquad (3)$$

where $L = |\bar{C} - \bar{D}|$

or perhaps $L = C_{\max} - D_{\min}$

The multiplicative constant for U is now restricted by the number of states that we can reliably discriminate between. We can put any interpretation we like on 'reliably', but we suggest a confidence limit of, say, 99 per cent (where $P < 0{\cdot}01$). If we wish to make our sets of relative similarities comparable to one another, then the important criterion is to have comparable Ps in the different sets. By being conservative we can also minimize the likelihood of overweighting similarities where P is calculated, compared to similarities where P is judged intuitively. The confidence limit of the mean of a number of measurements is related to the unit standard deviation, so that we could calculate the undifference at $P = 0{\cdot}01$ for an identical pair of means $\bar{A}$,$\bar{A}$, on a scale of length L. However, this comparatively elegant method is computationally difficult and probably unwarranted for the usual number of measurements used to compute values for states. Also, we will usually have a number of sets of similarities where U is judged intuitively. When the measuring error is approximately constant over the given range of values (case 1*a*), we would prefer to assume that P will be sufficiently small if we let $A_{\max}$ be the highest measurement for A, and $A_{\min}$ the lowest,

and $R_A = A_{\max} - A_{\min}$

and $\overline{R} = \dfrac{R_A + R_B + \ldots R_i \ldots + R_n}{n} \qquad (4)$

and $U = \dfrac{L}{\overline{R}} \approx U_{AA} \approx U_{BB}$

When we estimate, or judge, the states of a non-numerical attribute, and distribute them among a number (n) of ordered classes that are intuitively equally reliable, then the undifference of the system, U, is given by the number of class intervals ($n - 1$), and this may be taken as the scale length, L, for use in computing similarity. Since n and U depend on the number of equally reliable (but not necessarily equally probable) states that an attribute can exhibit, we suggest that they are measures of the relative information content for classification. If we wished to sum sets of similarities to obtain an overall similarity, then the one dimensional similarities could be appropriately weighted according to:

$$Ws_{AB} = \log_2 (U + 1) = \log_2 n \qquad (5)$$

For measured states, where the measuring error is not constant, but is a function of the value of the mean (case 1*b*), one procedure would be to compute a separate U for each pair of states by assuming that the relation:

$$U = \tfrac{1}{2}(U_{AA} + U_{BB}) \qquad (6)$$

holds good. This would mean, in effect, that the similarities in one relative set would be based on different numbers of classes and would have different weights. To avoid this intuitively unsatisfying arrangement, and to simplify computation, it would seem better to transform the scale used, so that the error in scale units is independent of the mean of the state. We could then proceed as for case 1*a*. For estimated or judged states, we would intuitively compensate for value-dependent errors when we form equally reliable classes.

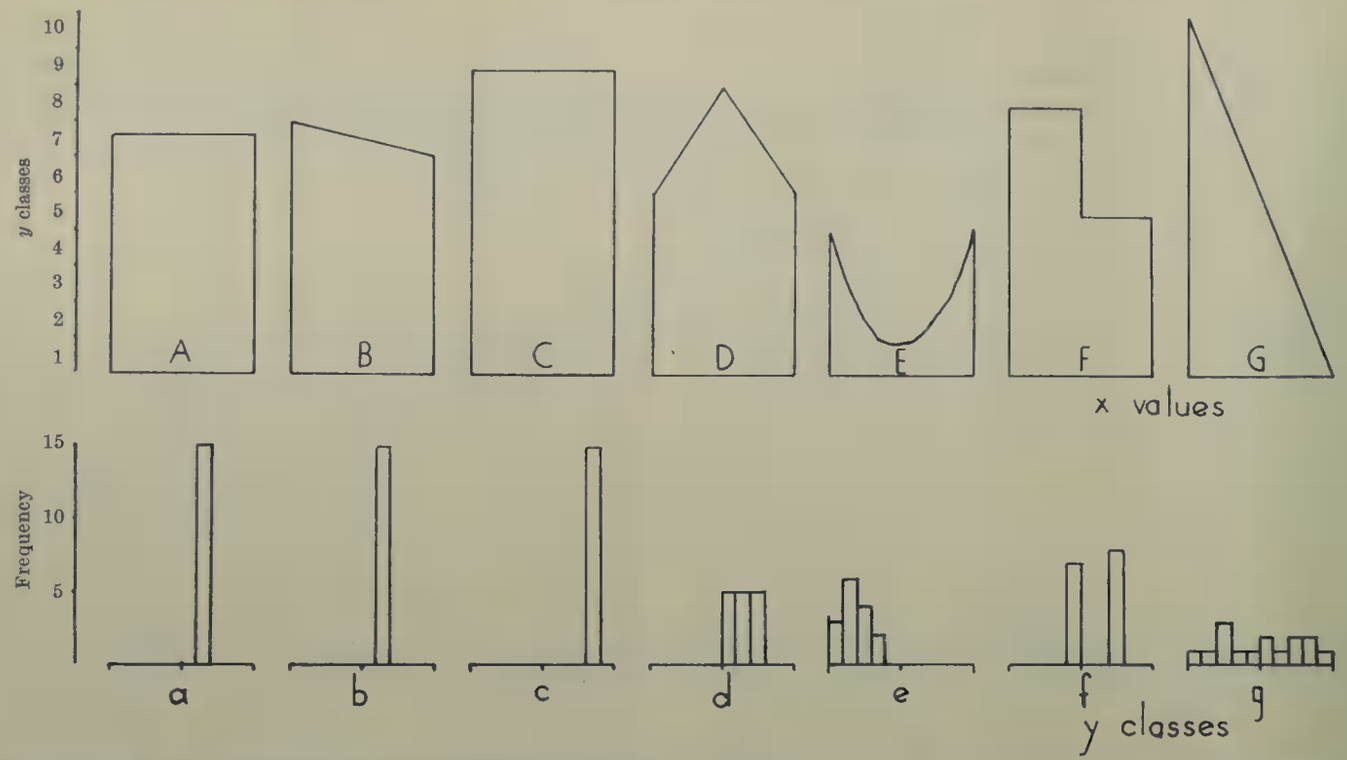

Fig. 1. *A–G.* Models of an attribute (*y*), in which the state varies over another dimension (*x*). *a–g*, Histograms derived by taking 15 measurements of *y* randomly distributed over *x*

When the spread of values, on repeated measurement, is larger than we would expect the measuring error to be, we would assume that non-unidimensionality of the attribute is the cause. Let us assume that the error in the measuring system can be estimated. We can then make a scale of a number of classes of equal confidence and small P. If repeated measurements fall into more than one class, lack of unidimensionality is present (case 2). To illustrate this point, consider the irregular bars in Figs. 1*A–G*. If we assume that each of these bars has a height, in arbitrary classes on the y axis, and we repeatedly measure the height at random over the x dimension (axis), we will get the histograms shown in Figs. 1*a–g*. The shapes of the bars define the histograms; but if we do not know how the y measurements are distributed over the x dimension (even whether they are random or not), the histograms do not uniquely define the shapes of the bars (for example, where y is spore-length and x is the reason(s) why different spores are not the same length).

Following our previous rationale, the procedure in this case would be to compute similarities on the means for the y measurements, and the undifference as in equations (4).

Another, more presumptuous, approach would be to assume that the distribution of frequencies in the histograms had some meaning which should affect the similarities as well as the weights. With this assumption we could compute a similarity based on the undifference of each measurement in each class of A compared with each measurement in each class of B. To compute this kind of similarity we would: let the state of A be an array of frequencies, $A_1, A_2, A_3 \ldots A_i \ldots A_n$; and the state of B be an array of frequencies, $B_1, B_2, B_3 \ldots B_j \ldots B_n$; and the sum of the frequencies of A be called F_A; and the sum of the frequencies of B be called F_B; and the range or scale-length, $n-1$, be called L. Then the similarity of A and B is given, when $F_A = F_B$, by:

$$S_{AB} = \frac{\sum_{i,j=1}^{n} A_i \cdot B_j (L - |i-j|)}{\frac{1}{2}\left[\sum_{i,j=1}^{n} A_i \cdot A_j (L - |i-j|) + \sum_{i,j=1}^{n} B_i \cdot B_j (L - |i-j|)\right]} \quad (7)$$

or rewriting the above equation in simpler notation,

$$S_{AB} = \frac{U'_{AB}}{\frac{1}{2}(U'_{AA} + U'_{BB})} \quad (8)$$

When $F_A \neq F_B$, we can correct by expanding to:

$$S_{AB} = \frac{U'_{AB} \cdot F_A \cdot F_B}{\frac{1}{2}(U'_{AA} \cdot F_B^2 + U'_{BB} \cdot F_A^2)} \quad (9)$$

This equation maintains the general relation that similarity is the fraction of the range not included between the states, but both the 'range' and the 'distance' between the states are affected by the spread of the values about the means of the states. Secondly, this equation stipulates that frequencies-in-common, or close frequencies, are more important in contributing to similarity than more widely separated frequencies are important in detracting from similarity. The equation does not assume a normal distribution of frequencies, and the results with skewed distributions are intuitively satisfying. The high similarities yielded with very broad spreads or bimodal distributions (because of the second effect already noted) are somewhat disturbing intuitively. The generally higher similarities that result when we use frequencies, rather than means of single values, are reasonable enough, since the similarity is still zero when all values fall in the two most distant classes.

Not only does increasing spread result in increasing similarity, but the spread of the states results in an effective compression of the range and this should be recognized in computing the weighting of the similarity. This is easily accomplished by modifying equation (5) so that:

$$W = \log_2\left(n.\frac{\frac{1}{2}(U'_{AA} + U'_{BB})}{U'_{\max}}\right) \quad (10)$$

where $U'_{\max} = F_A \cdot F_B \cdot L$

which, if $F_A = F_B$ simplifies to:

$$U'_{\max} = F^2 \cdot L$$

When we know the way in which the y measurements are distributed over the x dimension, we would probably not want to use either of the foregoing methods. For example, if y represents growth rate and x represents different known temperatures, one could compute a similarity for the growth rate at each temperature used. How many temperatures (values of x) should we use to

compute an overall similarity for growth rate (state of y)? Obviously the range of temperatures is limited by the lowest and highest temperatures at which any of the strains will grow (that is, the only useful values of x are those for which not all the y values are identical). The number of temperature values, within this range, which would contribute new information about the distribution of y over x, is limited by the minimum temperature difference which would produce growth rates between which we could reliably discriminate. Thus, we have a finite number of test classes, as well as attribute classes, and the number in each case is based on equally reliable discrimination within the range found. If we compute a set of similarities for an attribute, in each of a number of test classes, in each of a number of test dimensions, then we would need a weighted mean similarity for the attribute under all test conditions in order to compare it with, or add it to, similarities for other attributes. The relative weight of such mean similarities for single attributes should be the log of n for the whole attribute-test system, rather than the sum of the log n's in each test class of each test dimension (compare equation (5)).

We have promulgated a principle for determining similarities in one dimension and devised some methods for applying it to attributes to yield sets of relative similarities and weights. An obvious way to produce, from these, sets of overall similarities ($\bar{S}_{AB}$'s) is to let:

$$\bar{S}_{AB} = \frac{\sum_{i=1}^{n} S_{AB_i} \cdot W_{AB_i}}{\sum_{i=1}^{n} W_{AB_i}} \qquad (11)$$

The weights of the overall similarities should be the sums rather than the averages of the weights for the individual similarities.

The matrix of weighted, overall similarities then needs to be transformed into a useful classification. One promising approach appears to be through multidimensional scaling[3-6]. This approach might even be able to compensate for the defect that the supposed one-dimensional similarities contain varying redundancy when summed.

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A.C. RESISTIVITY OF ANODIC OXIDE FILMS ON VALVE METALS

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THE frequency dispersions of a.c. resistivity of anodic oxide films on niobium, tantalum, aluminium, zirconium, titanium and tungsten have been measured and also the resistivity profiles through films on niobium by an extension of the technique of Heine and Pryor[1].

Specimens in the form of sheet or as the ends of wires were generally chemically polished, followed by a 10-sec etch in 40 per cent HF in the case of tantalum and niobium. Aluminium, however, was simply etched in 0·5 N NaOH solution for 15 min at 20° C followed by a 1-sec dip in 50 per cent HNO_3 at 90° C, and zirconium was finished on 1μ diamond suspension. The specimens were anodized, generally in 3 per cent w/v ammonium tartrate solution (pH adjusted to 7), at 10 m.amp/cm² to an appropriate cell voltage followed by current decay, or occasionally to a given overpotential. The frequency dispersions of impedance of as-formed films or of films thinned in 1 per cent HF were measured, generally in tartrate solution, using a General Radio capacitance measuring assembly type 1610-B with a 50-mV p–p signal. This permitted the calculation of resistivities, on the assumption of the validity of a simple electrical analogue for the film of a resistor in parallel with a capacitor at any one frequency. Such results are plotted as bold curves in the diagrams; dotted curves, where included, indicate the effect of correcting for double-layer resistance by the simple method of Heine and Pryor[1]. Thinning was judged to be macroscopically and microscopically uniform from interference colours and transmission electron microscopy respectively. As-prepared films seemed flaw-free and the flaw density was so low in thinned films that there appeared to be negligible effect on the impedance. The film capacitance was a sound measure of film thickness when compared with interference-colour determinations.

Fig. 1 shows the frequency dispersions of average a.c. resistivity for films 230, 1150 and 2300 Å thick formed on zone-refined niobium (purity better than 99·99 per cent except for about 100 p.p.m. tantalum) at 10 m.amp/cm², followed by appropriate current decay to a residual current of less than 20 μamp/cm². Clearly the thicker the film the higher the average resistivity. This could suggest either that the films become more perfect the thicker they grow, or that the resistivity of the film region adjacent to the metal is lower than elsewhere. Evidence presented here favours the latter interpretation. The observation seems to be a general one for valve metals, because it holds at all frequencies for tantalum and niobium in aqueous and non-aqueous solutions and for aluminium in non-aqueous solution. It is also true for aluminium and zirconium in aqueous solution at 10 kc/s and above. The curves converge at and below 1 kc/s for aluminium in aqueous solution, probably because of effects due to gross impurity substitution of OH^- ions[1,4], and for zirconium possibly for similar reasons. The phenomenon could not be checked for tungsten because the films thinned or showed strange behaviour during and immediately after formation in many electrolytes, or for titanium because a sufficient range of film thicknesses could not be obtained. For niobium, and particularly for tantalum, the results were relatively independent of metal purity (99·8 per cent to zone-refined), anodizing electrolyte and whether the main anodizing current density of 10 m.amp/cm² was terminated abruptly or by current decay.

Figs. 2a and b give the average resistivities at 1 kc/s and 100 kc/s respectively, measured after various stages of thinning by 1 per cent HF, for a 2300 Å film formed on zone-refined niobium. Each point plotted represents the average value for the film of that thickness remaining on the specimen, not the resistivity of a small element of oxide in that location[1]. Results for 230 Å films thinned in 1 per cent HF and in M.Na_2CrO_4 solution with pH adjusted to 11·2 with NaOH solution (representing a much slower thinning rate) were quite similar. The various results indicate low-resistivity oxide adjacent to the metal at both frequencies, confirming the trends reported here. According to the Heine–Pryor theory[1], this represents an n-type semi-conducting region containing oxygen ion

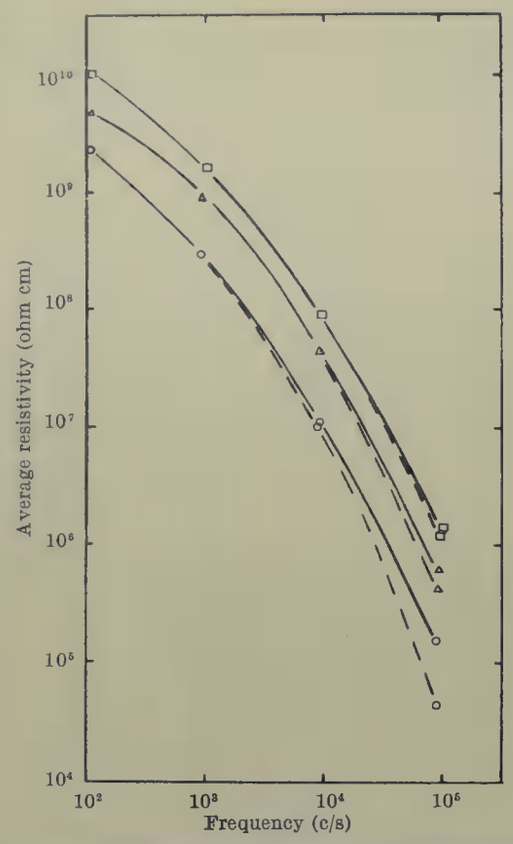

Fig. 1. Frequency dispersions of average resistivity for anodic oxide films on niobium. ○, 230 Å; △, 1150 Å; □, 2300 Å

vacancies or possibly excess metal ions. The associated partly trapped electrons required for electrical neutrality produce principally electronic relaxation effects observable at both frequencies. Presumably this represents a steady-state situation because it is held[2] that about 70 per cent of Frenkel defects present during formation rapidly disappear on stopping the current. When the results at 100 kc/s, corrected for double-layer resistance, are plotted as equivalent parallel resistances, R_p (obtained by multiplying the resistivity by the thickness at each thickness), a smooth non-linear curve is obtained. If R_p is plotted against the square of the thickness, a straight line passing through the origin is obtained, indicating that the number of these n-type defects varies with the inverse distance from the metal/oxide interface for the entire film, according to the theory of Heine and Sperry[3]. This contrasts with films on aluminium where the metal-deficient region extends only 60–80 Å into the film for 240 Å films[1,4]. These preliminary results require confirmation, but the difference might be due to the greater solubility of oxygen in niobium. The undulations in the outer regions at 1 kc/s may be genuine because they are semi-quantitatively reproducible, but further work is required to explain them. The initial drop was originally thought to be due to opening of flaws by the HF, but simple calculations of the contribution of conduction down the few flaws to the overall impedance do not support this argument. The average resistivities at both frequencies for films thinned to 1150 Å and 230 Å respectively agree within experimental error with the corresponding values for films formed only to these thicknesses. If films on all the valve metals could be thinned uniformly (which is doubtful), it is probable that resistivity profiles of the general form shown would always be found. Similar, but not identical, curves have been obtained for aluminium in non-aqueous solutions[4], which should prove more amenable to quantitative interpretation than the more complex ones obtained on partially hydrated films formed in aqueous solutions[1,4].

Fig. 3 presents a comparison of the frequency dispersions of average a.c. resistivity of 230 Å films formed on the various metals. Table 1 lists important constants for the metal oxides obtained from many sources. The values of dielectric constants used to calculate the appropriate

Table 1. Important Constants for Anodic Oxide Films on Metals

Metal and oxide	Dielectric constant	Melting point of bulk oxide (° C)	ϱ Factor (metal ion radius)/(oxygen ion radius)	Refractive index	Å/V ratio
Aluminium (Al_2O_3)	8–10·5 (8)	2,050	0·38–0·39	1·52–1·67	10–14 (12)
Zirconium (ZrO_2)	22–24·7 (22)	2,700	0·60	2·13–2·2	21–25·6 (25)
Tantalum (Ta_2O_5)	27·6	1,870	0·52–0·56	2·12–2·3	17·5
Niobium (Nb_2O_5)	41·4	1,520	0·52–0·54	2·22–2·46	23
Tungsten (WO_3)	41·7	1,473	0·47	2·2	18
Titanium (TiO_2?)	12–114 depending on form of TiO_2 (107)	1,640	0·52	2·5–2·9	20–50 (20)

resistivities and the Å/V ratios used to estimate film thicknesses are given in brackets where a range of values is quoted. Clearly, the lower the dielectric constant of the film the higher its a.c. resistivity. In order to obtain the curves presented it was necessary to know the dielectric constants of the films and their Å/V ratios. The only case of uncertainty is titanium, where both parameters are not well defined and a variety of curve positions can be obtained. The curve for tungsten (not included) lies near that of niobium (similar dielectric constant) but rather below it because the film tends to dissolve. Now, Young[5]

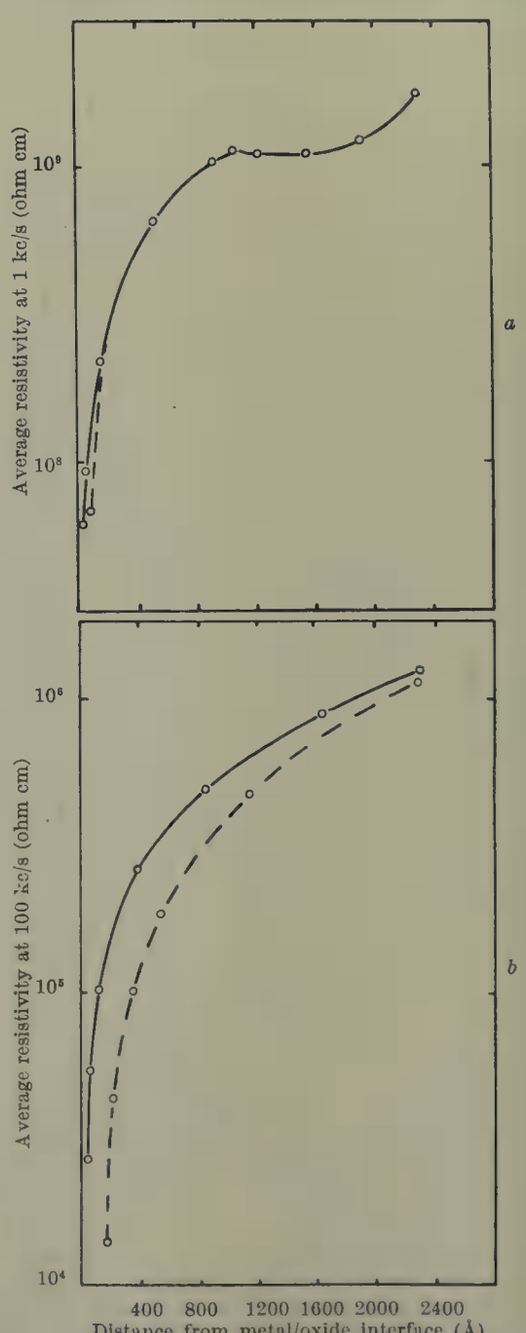

Fig. 2. Average resistivity measured during thinning of 2300 Å film formed on niobium. *a*, 1 kc/s; *b*, 100 kc/s

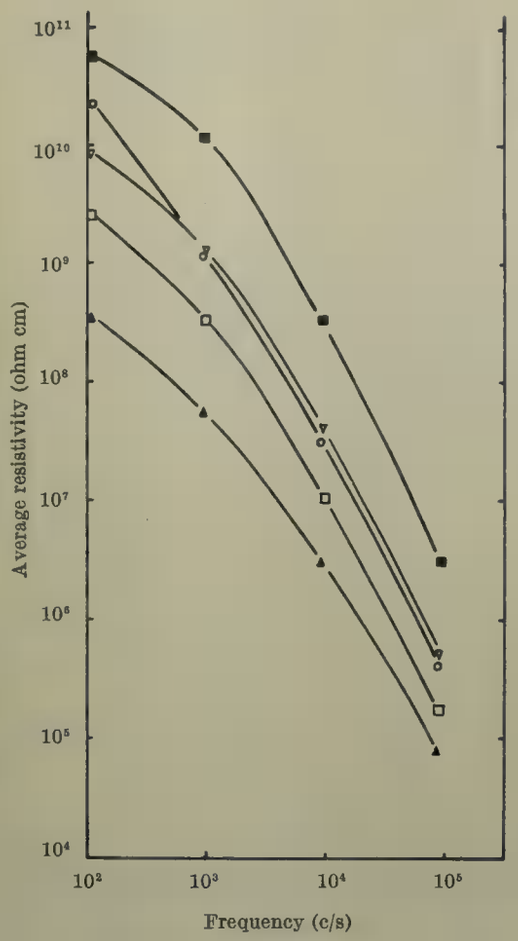

Fig. 3. Comparison of frequency dispersions of average resistivity for 230 Å films formed on various metals. ■, Al; ▽, Zr; ○, Ta; □, Nb; ▲, Ti

has suggested that the higher the dielectric constant of the film, the lower its ionic resistivity. Although the a.c. resistivity measured with a low applied alternating voltage is complex, probably receiving contributions from several conducting processes, and is not necessarily directly related to the ease of ion movement under forming conditions at high direct voltages, the same trend seems to hold.

High a.c. resistivity (low dielectric constant) appears related to high oxide melting point (refractoriness), with titanium and zirconium as exceptions. The melting points quoted are, of course, for bulk oxides and do not necessarily apply to thin anodic films which are generally neither pure nor fully crystalline. The curve for the anodic film on zirconium may be out of position on the melting point criterion due to the film's greater tendency towards crystallinity. In any case, the thin-film, glass-forming characteristics (apparently favoured by a small ratio of metal ion radius to oxygen ion radius (ρ factor) but not well understood[5]) may be important and modify any criterion related to bulk melting point alone. Thus, Al_2O_3 would be expected to be a better glass-former than ZrO_2 on the basis of ρ factor, although it does not vitrify readily in the bulk state, and this may partly explain their relative resistivities. Apparently Ta_2O_5 and Nb_2O_5 should have good glass-forming characteristics but may be restrained from acting in this way by high ρ factors[5]. The low resistivity of TiO_2 is said to be more likely due to poor glass-forming characteristics than to impurities[5]. However, anodic films on zirconium and titanium are notoriously leaky compared with those on other metals, which may account for their behaviour. There appears to be little direct trend of a.c. resistivity with either refractive index or Å/V ratio, which is not surprising.

Very recent consideration of the foregoing results, in the light of apparently more precise film and double-layer equivalent circuits, methods of determining point resistivities through the film and of applying the double-layer impedance correction, indicates that there may be a need to modify the absolute values of resistivity given here. Because of their complexity, these ideas will have to be elaborated in detail elsewhere[6]. Most of the foregoing trends still hold, but the theory based on the simple model may require some modification in due course.

We thank Prof. T. K. Ross for providing facilities and the Science Research Council for a maintenance grant to one of us (C. P.).

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COLOUR OF 'PURE' WATER*

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In a previous article[1] a method was described for obtaining a fundamental specification of the colour of ocean or lake water, independent of the spectral variation of the natural lighting and independent of the spectral sensitivity of the photodetector.

The method consists essentially of three steps: first, the choice of suitable geometry for measuring the input flux to the body of water and the output flux from the water to the instrument; second, the measurement of a fundamental spectrophotometric property of the water body (which has been called the output/input ratio); and, third, the computation from these data of the trichromatic coefficients of the water colour. This method has recently been used to determine the colour of the exceptionally clean water of Crater Lake.

* The results used in this article were obtained during the course of research on optical methods for *in situ* detection and estimation of chlorophyll and other pigments in the ocean, a research programme which is supported by the U.S. National Science Foundation, Earth Sciences Division.

Crater Lake is the principal feature of Crater Lake National Park, a U.S. National Park in South-western Oregon. The lake is 20 miles in circumference, about 2,000 ft. deep, with its surface at an altitude of about 6,000 ft. It has no inlet other than rain or melting snow. The surrounding crater wall is sparsely vegetated and sparsely populated by wild animals, so that contamination from these sources has been small. One of the outstanding characteristics of the lake is its unusual blue colour, which is, of course, a consequence of its lack of contamination.

In 1940 the lake was examined for phytoplankton and particulate matter by Utterback and Phifer[2]. They found that, "In the upper 20 metres of the lake, there was practically no phytoplankton . . .". They also noted that the water contained small particles of volcanic glass, but they did not undertake to determine the vertical distribution of these latter particles.

In this work, the colour of Crater Lake was measured with the same instrument used to obtain the data of the previous publication[1]. The instrument was mounted underneath a small raft with the irradiance plate for measuring the input flux to the water just above the water surface, and the axis of the underwater collection system oriented horizontally. Generally clear, sunny weather prevailed during the measurements.

The average output/input ratio data for Crater Lake, shown in Fig. 1, were obtained from a location off an island near the west side of the lake. The instrument was oriented to point in a north-westerly direction in order to avoid the forward scattered flux from the Sun's direct rays.

Although it is valid to specify the colour of the water for any chosen direction, it is more difficult to obtain a satisfactory measurement in the Sun's direction because the forward scattered component of the sunlight rapidly changes in magnitude as the Sun changes its position relative to the instrument's direction of observation. With the Sun behind the instrument, repeated measurements taken an hour or more apart showed no significant changes in the output/input ratio due to the changes in the Sun's position.

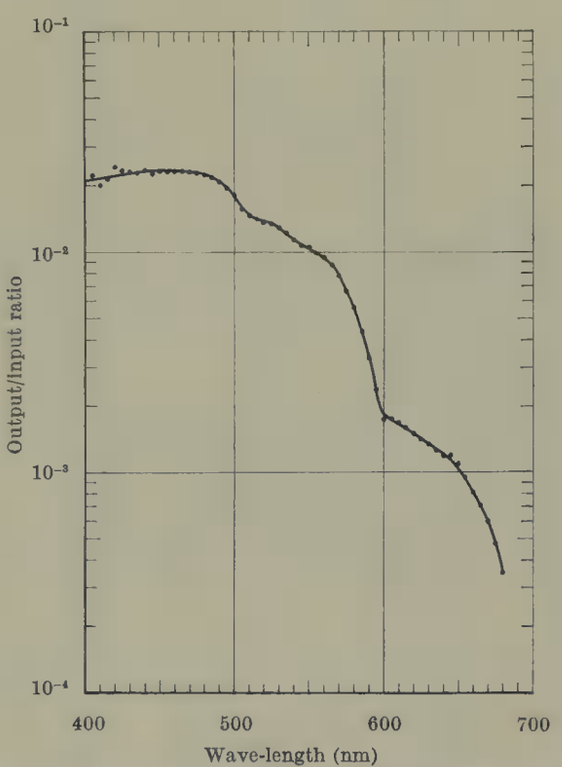

Fig. 1. Average output/input data for Crater Lake (August 23, 25, 1964)

The data of Fig. 1 have been used, together with the relative spectral distribution of the energy in sunlight above the Earth's atmosphere, to calculate the tristimulus values of the colour of Crater Lake water. This colour has been plotted in the C.I.E. co-ordinate system in Fig. 2, and from the plot the following colorimetric specification has been obtained:

Dominant wave-length:	483 mμ
Photometric brightness:	91·7 per cent
Purity:	56·0 per cent

One of the interesting features of this method for determining the colour of the ocean or a lake is the ability to make accurate comparisons of the colour of remotely separated water bodies. The cleanliness of Crater Lake can be immediately deduced from Fig. 2, and the extent of the chlorophyll contamination in the other waters illustrated can be comparatively judged. If data were available, it should also be possible to distinguish

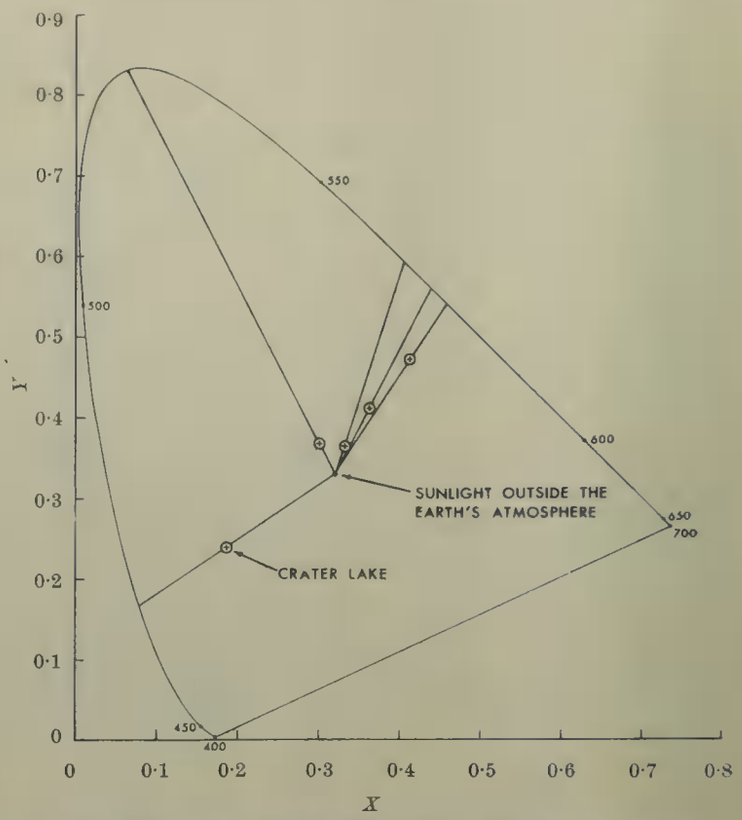

Fig. 2. C.I.E. plot to show colour of Crater Lake water relative to sunlight outside the Earth's atmosphere as a reference standard. The other points represent water containing chlorophyll (see ref. 1)

red algae from green, since these colours would occupy characteristic regions of the diagram.

One objective of this work was to obtain output/input data on the purest water that could be found. Such data could then serve as a standard, against which to estimate the contamination of other waters. There are, no doubt, many bodies of clean water which will compete for the 'honour of being the purest', and further measurements will have to be made if the ultimate standard of purity is to be found.

It is possible, however, to make one interesting comparison. In 1930, Beebe[3] published a paper in which he described the spectra of the spacelight observed in a horizontal direction under water. Beebe's observations were visual, and consequently must be interpreted with care, since, for the greater depths at which he worked, his vision was scotopic and the spectral sensitivity of his eye would have been shifted considerably. On the other hand, at a depth of 800 ft., the selective absorption of light by water must have converted ordinary daylight into nearly monochromatic light. If we assume that this was the case, then we can deduce from Beebe's observations that the minimum value of the attenuation coefficient for Bermuda water occurred at 520 mμ.

From Fig. 1, the minimum value of the attenuation coefficient for Crater Lake is at about 450 mμ. It follows, therefore, that Crater Lake water in 1964 was bluer and cleaner than was the water off Nonsuch Island in 1930.

For the detection and estimation of specific pigments such as chlorophyll, it is of course more useful to work directly from the output/input data rather than from the colour of the water. References to papers on this subject are given in refs. 4 and 5.

I thank the staff of Crater Lake National Park, especially R. A. Nelson, superintendent, Donald Robinson, assistant superintendent and R. M. Brown, chief park naturalist, for their help.

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RELATIONSHIP BETWEEN THE ELECTROMYOGRAM AND THE FORCE OF THE ISOMETRIC REFLEX RESPONSE OF NORMAL HUMAN SUBJECTS

By ALEX M. CLARKE*

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EARLY research by physiologists revealed a correlation of electrical (motor unit) activity with muscular contraction in spinal and decerebrate animals as well as involuntary contractions in man[1]. Since then many reports have appeared in the literature to show that surface electromyograms (EMG) bear a linear relationship to the magnitude of voluntary muscular tension under conditions of isometric contraction[2–4]. However, no observations appear to have been reported of a relationship between the magnitude of the isometric response in a myotatic stretch reflex and EMG in man.

In the experiments described here, the knee-jerk was selected for examination for the following reasons: (*a*) the neural linkage for the α-route at the lumbar region of the spinal cord is monosynaptic, thus providing a reasonably simple pathway[5–7]; (*b*) the response is remarkably restricted in scope at low levels of excitation[8]: the reaction is confined to the quadriceps femoris, while synergist and antagonist muscles usually are inhibited or unaffected; (*c*) the subject can sit and relax in a chair specially designed to hold him in a comfortable, standard, symmetrical position since body and limb positions are known to affect reflexes[8,9]. There is adequate evidence to establish the functional dependence of the myotatic stretch reflex on the excitation of the primary (annulo-spiral or nuclear bag) ending of the muscle spindle which is exclusively innervated in mammals by small-diameter motor nerve fibres (γ-efferents)[5,10,11]. In this way feed-back is provided which, although being too slow to monitor reflex movements, has a strong indirect influence on the spindles, biasing them for different tensions of extrafusal muscle and rates of movement[12,13]. The γ-efferent system itself is regulated from a number of central nervous system structures, notably the mesencephalic, diencephalic and bulbar regions of the reticular formation[14]. In addition, γ-discharge has been found to relate closely with change in level of cortical activity so that spindle sensitivity, generally speaking, is controlled centrally by the degree of alertness or activation of the subject[15,16].

Therefore, in an attempt to reduce cerebral activity in the expectation of making γ-discharge at least more consistent than it is in normal circumstances, the three young normal male adults who were used in the experiments were trained to apply Jacobson's method of mental and physical relaxation[17]. At least six daily sessions of 1 h each were required to train the subject individually to reach the criteria of displaying: (*a*) quiescent EMGs, indicating supported and relaxed muscles before and after the mechanical tap to the ligamentum patellae[18]; (*b*) discrete, highly synchronous muscle action potentials in response to the stimulus[19].

Figs. 1 and 2 show comparisons between the records obtained by trained and untrained subjects.

Testing was conducted in a sound-proofed, electrically shielded and thermally insulated room in order to control auditory and visual stimuli, room temperature and relative humidity, and to suppress 50 c/s electrical interference for EMG purposes[20]. Silver disk surface electrodes, 11 mm diameter, with a shallow central depression were applied in standard positions 3 in. apart over the belly of the muscles, rectus femoris and biceps femoris. EMG signals were amplified through push-pull circuitry and fed to a Both pen recorder. Force records were obtained by amplification of signals from silicon strain gauges mounted on the pendulum-hammer and on the ankle cuff linkage.

Peak forces between 1·5 kg and 3·5 kg were applied to the ligamentum patellae by varying the height from which the hammer was dropped in some sessions and, on other occasions, the hammer was dropped from a standard height for a series of trials. Under the latter conditions, it was found that subjects could maintain consistent muscle tonus over short periods involving about ten taps as indicated by uniform force records.

However, irrespective of whether there were variations in the force applied to the ligament (implying variations in the extension of the muscle), slight changes in muscle-resting tension, or consistent muscle tone, between trials

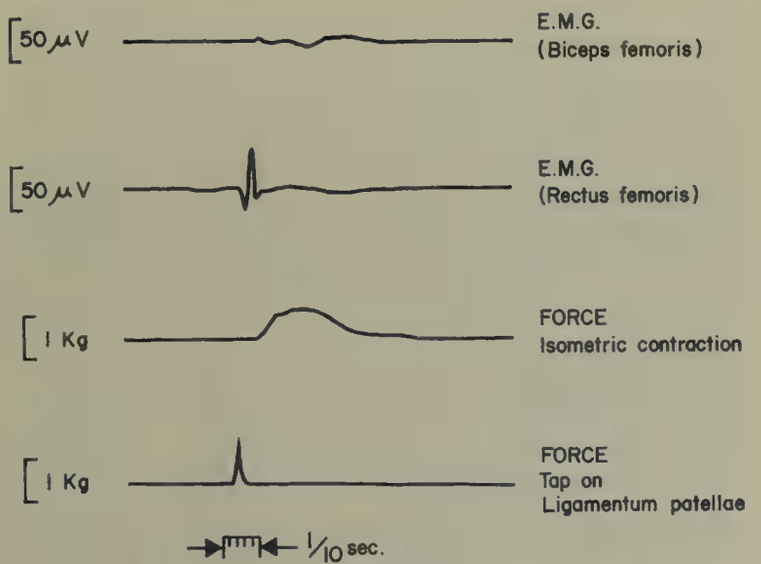

Fig. 1. Simultaneous electromyograms and force records showing electrical quiescence in the muscles of a trained and relaxed subject (G. B.). The discrete, diphasic potential in the EMG (rectus femoris) is the result of the tap on ligamentum patellae and indicates a high degree of synchrony in the discharge of muscle action potential. Negligible activity in biceps femoris

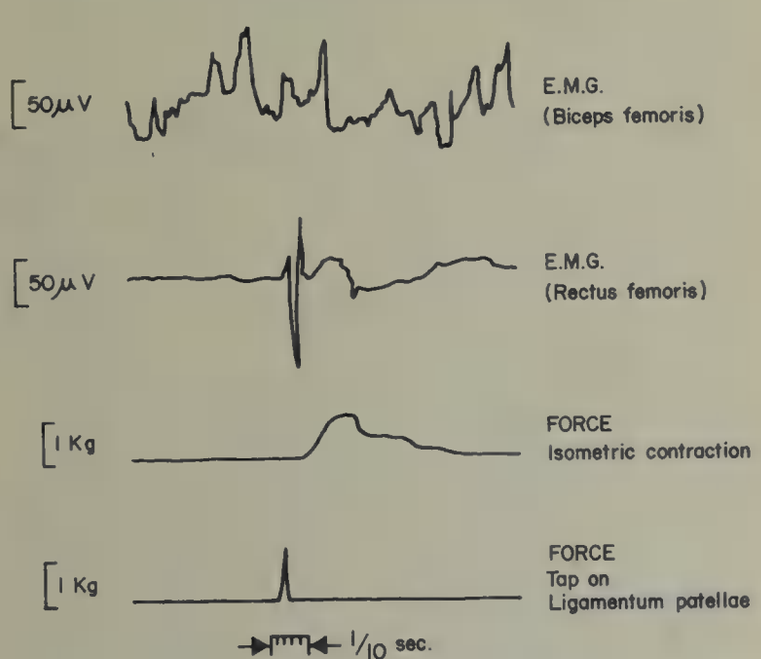

Fig. 2. Simultaneous electromyograms and force records showing random electrical activity in the muscles of an untrained and tense subject (A. M.). Hump in the force record of the isometric contraction indicates that a clonus condition has occurred. The 'interference pattern' EMG is evident in biceps femoris

* Commonwealth postgraduate research scholar.

Table 1

Subject	Session	N	Isometric response impulse (g sec) $\bar{x}$	Isometric response impulse (g sec) S	Electromyogram electrical impulse (μV sec) $\bar{x}$	Electromyogram electrical impulse (μV sec) S	Product moment correlation*	Confidence limits of 99% for $r_{pop.}$ (Z transformation of R. A. Fisher)
A. M.	1	15	231·1	63·9	2·83	0·27	+0·978	0·995 to 0·914
	2	21	196·3	32·1	4·17	0·65	+0·918	0·975 0·753
	3	21	315·1	90·6	3·25	0·98	+0·933	0·979 0·782
	4	18	268·8	108·9	3·07	1·29	+0·943	0·984 0·790
	5	33	276·7	105·1	2·10	1·37	+0·979	0·992 0·949
	6	25	269·3	115·2	3·19	0·81	+0·890	0·962 0·702
	7	25	255·9	89·9	1·84	0·95	+0·960	0·986 0·885
	8	14	394·6	113·1	2·02	0·93	+0·977	0·995 0·885
G. B.	1	19	137·5	31·3	3·76	0·82	+0·918	0·977 0·736
	2	16	244·9	39·1	5·99	1·18	+0·967	0·993 0·879
	3	21	156·9	19·7	2·12	0·57	+0·923	0·979 0·759
	4	24	128·6	42·9	1·76	0·95	+0·934	0·978 0·811
	5	16	243·6	23·8	4·09	0·78	+0·812	0·952 0·400
	6	16	300·1	46·8	3·88	1·10	+0·946	0·986 0·789
	7	26	168·6	30·6	1·89	0·59	+0·897	0·960 0·726
	8	37	371·3	69·8	2·65	0·93	+0·935	0·975 0·861
	9	35	324·3	69·1	1·94	0·75	+0·875	0·949 0·726
	10	32	303·1	66·7	1·33	0·51	+0·882	0·952 0·716
W. R.	1	19	103·8	35·8	2·28	0·60	+0·909	0·974 0·707
	2	12	111·3	33·0	2·28	0·60	+0·923	0·985 0·623
	3	17	227·9	45·4	1·58	0·61	+0·969	0·992 0·885
	4	26	172·4	53·9	3·55	0·57	+0·904	0·968 0·757
	5	25	176·4	78·6	5·07	0·87	+0·841	0·945 0·758
	6	14	148·9	39·3	2·45	0·26	+0·897	0·977 0·592
	7	27	79·4	64·6	2·94	0·61	+0·975	0·991 0·932

* All correlations are significantly different from zero ($P < 0·001$).

in any one session, a high positive relationship was found between the impulse (the integral of force over time) of the isometric contraction, measured at the ankle, and the EMG impulse of the quadriceps muscle.

Table 1 provides data relevant to the product-moment correlations obtained between these two variables for the three subjects.

The explanation for this near linear relationship probably lies in the consistency of γ-innervation of the spindles under the experimental conditions imposed for these subjects and in the fact that there was no complicating activity from the flexor muscles. The γ-discharge causes the contraction of the intrafusal fibres and leads to a decreased threshold of the muscle spindle to stretch[21]. It is thought that in many instances, a reflex is augmented by an increase in γ-efferent activity[22]. Thus, fluctuations in the sensitivity of the spindles to stretch by variable activity of the γ-motoneurones in biasing the intrafusal muscle fibres would result in a greater or lesser number of spindle receptors firing when stimulated by the tendon tap at any given time. Assuming that the motor end-plates of the extrafusal fibres fire proportionally to the number of spindles activated, then one may take the discrete, highly synchronous potential impulse of the EMG as the best, indirect indicator of the magnitude of γ-biasing of the spindles of the quadriceps muscle (rectus femoris). In view of the small standard deviations obtained in this variable, one might conclude that fluctuations in small fibre control of the stretch reflex response were minimal.

Some preliminary work on the correlation between the maximum force of the isometric contraction and the impulse has yielded correlations of the order of +0·992 which probably indicate that the reflex response is ballistic. In this event, the γ-innervation would be too slow to have any direct effect during the response; presumably the force-time relations of the muscle contraction under isometric conditions are programmed beforehand by at least such factors as the degree of sensitization of the spindles and the initial resting tension of the muscle. Triggering occurs when the mechanical tap imparts a sudden, brief stretch to the muscle.

Consequently, provided that there is no modification of the knee-jerk response by involvement of flexor muscles and if fluctuations in γ-control over spindle sensitivity are minimal, a high positive relationship would be expected between the EMG and the isometric contraction of the extensor muscles.

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PURINE RICH REGULATORY SEQUENCES IN MESSENGER RIBONUCLEIC ACID

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THE elegant proposal for the existence of a messenger RNA by Jacob and Monod[1] has been supported by evidence obtained from phage-infected cells[2–5], step-down transition cells[6–10] and log phase bacterial cells[3,10,11]. The results from these systems indicated that a polyribonucleotide fraction had most of the properties postulated for a messenger RNA (*m*-RNA), that is, a short half-life[6,12], a DNA-like base composition[6,10], a heterogeneous size[13,14], an affinity to ribosomes[15,16] and a sequence complementarity to DNA[4,6]. However, there is still a paucity of results regarding the actual template function for protein synthesis of the *in vivo m*-RNA fraction. The most definitive results have been obtained by using synthetic polyribonucleotides[17,18] or phage RNA[19,20] as messages. Synthetic polymers have been used for incorporation of specific amino-acids into polypeptides. The product made in the presence of phage RNA has polypeptides ordinarily found in the phage coat protein. In binding experiments, polynucleotides[21–23] and trinucleotides[24] attached to ribosomes have directed the binding of specific aminoacyl-transfer RNAs. These various studies have led to the present dogma that transfer of information from DNA to protein occurs through an *m*-RNA.

In this rapid advance in our knowledge of protein synthesis, it was felt that a critical analysis of the data available would produce some useful information on the

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Table 1. Base Ratios of Messenger RNAs

Organism	DNA %GC	*m*-RNA Moles per cent C	A	U	G	%AG	A/U	G/C	Ref.
B. subtilis	43	20·4	28·6	28·3	22·5	51·1	1·01	1·10	9
B. subtilis	43	20·5	31·0	26·3	22·2	53·2	1·18	1·08	9
B. subtilis	43	21·2	30·2	28·1	20·5	50·7	1·07	0·97	43
P. vulgaris	37	19·8	30·1	29·2	20·9	51·0	1·03	1·06	44
P. vulgaris	37	22·7	26·7	23·0	27·6	54·3	1·16	1·22	44
E. coli	50	19·7	26·6	28·9	23·4	50·0	0·92	1·19	45
E. coli	50	23·0	26·4	26·6	24·0	50·4	0·99	1·04	45
E. coli	50	23·5	25·9	25·4	25·2	51·1	1·02	1·07	45
E. coli	50	23·6	25·1	23·8	26·5	51·6	1·05	1·12	45
E. coli	50	24·9	25·1	23·8	26·2	51·3	1·05	1·05	45
E. coli	50	26·1	23·4	20·2	30·3	53·7	1·16	1·16	45
E. coli	50	27·3	22·1	20·2	30·4	52·5	1·09	1·11	45
P. aeruginosa	66	25·9	22·4	23·4	28·3	50·7	0·96	1·09	6
P. aeruginosa	66	27·0	21·5	21·8	29·7	51·2	0·99	1·10	6
P. aeruginosa	66	30·3	20·9	19·0	29·8	50·7	1·10	0·98	6
S. cerevisiae	36	21·7	28·6	28·7	21·2	49·8	1·00	0·98	7
S. pombe	—	20·6	33·8	23·6	22·0	55·8	1·43	1·07	8
S. pombe	—	17·8	35·0	23·8	23·4	58·4	1·47	1·31	46
A. punctulata	39	23·1	28·9	24·4	23·6	52·5	1·18	1·02	47
A. punctulata	39	22·8	28·1	27·4	21·8	49·9	1·02	0·96	47
HeLa cell	43	24·2	26·6	27·7	21·5	48·1	0·96	0·89	48
HeLa cell	43	26·9	25·4	27·3	21·0	46·4	0·93	0·78	48
β3 phage	43	17·6	33·2	26·4	22·8	56·0	1·26	1·30	49
α phage	44	20·5	31·0	23·0	25·5	56·5	1·35	1·24	32
T2 phage	34	17·4	31·0	28·7	21·9	52·9	1·08	1·26	44
T2 phage	34	22·8	23·0	21·2	33·1	56·1	1·08	1·45	2
T2 phage	34	22·4	24·6	22·0	31·0	55·6	1·12	1·38	2
T4 phage	34	15·4	30·6	32·9	21·1	51·7	0·93	1·37	50
T7 phage	47	22·6	27·4	28·3	21·7	49·1	0·97	0·96	51
$\varphi X174$ phage	42	17·5	23·8	33·1	25·6	49·4	0·72	1·46	31
$\varphi X174$ phage	42	17·5	25·5	34·0	23·0	48·5	0·75	1·31	31

properties of *m*-RNA. A particular property of the *m*-RNA which could be analysed was the reported base compositions of the *m*-RNA fraction. Table 1 presents a list of organisms and the base compositions of the *m*-RNA fraction isolated from these organisms. Three criteria were used for inclusion of RNA base ratios in this Table. The RNAs were (*a*) hybridized to DNA[4,44,50,57], (*b*) synthesized after phage infection, or (*c*) synthesized during step-down transition. The RNAs studied were pulse-labelled or steady-state labelled with $^{32}PO_4^+$ and analysed after alkali hydrolysis. Most of the RNAs were hybridized with DNA and separated from the bulk of the cellular RNA by differential sedimentation, column fractionation, or membrane filtration before analysis. Those RNAs not hybridized with DNA were obtained under conditions in which undetectable or very low levels of ribosomal and transfer RNAs were synthesized. A fact to remember is that these *m*-RNAs are heterogeneous populations.

The properties of the *m*-RNA in Table 1 can be summarized as follows:

(a) *Percentage of guanylate + cytidylate (%GC) or resemblance to DNA composition.* For most *m*-RNAs there is a resemblance of the %GC to that of the DNA. However, for many of them the %GC is significantly different from that found in the DNA. For *E. coli*, for example, the range is from 43·1 to 57·7 per cent. In *P. aeruginosa* the %GC of the *m*-RNA is much lower than that of the DNA. The deviation is less marked with the phage *m*-RNAs.

(b) *Percentage of adenylate + guanylate (%AG) or purine content of M-RNA.* All the RNAs except that from HeLa had a %AG of 50 ± 2 per cent or higher. The *m*-RNAs have either an equivalence of purines and pyrimidines or are purine-rich in all the cases examined except one. Conversely one can state that *m*-RNAs are pyrimidine-poor. The DNA composition of the organisms analysed was from 34 to 66 per cent guanylate + cytidylate.

(c) *The adenylate–uridylate ratio (A/U).* In most of the RNA populations the A/U ratio is equal to or greater than 1·0. In an extreme case the ratio is 1·47. If one considers a 2·0 per cent molar ratio difference between A and U as insignificant in the base composition analyses, one finds that in all cases the content of A is equal to or greater than U in *m*-RNAs.

(d) *The guanylate–cytidylate ratio (G/C).* Once again the G/C ratio is equal to or greater than 1·0 in all cases except for HeLa cells.

Before discussion of the possible significance of these facts, it is of interest to consider viruses, since viral RNA

Table 2. Base Composition of Viral RNA

Virus	Moles per cent C	A	U	G	%AG	A/U	G/C	Ref.
Tobacco mosaic	18·5	29·8	26·3	25·4	55·2	1·13	1·37	52
Cucumber	18·2	25·7	30·6	25·4	51·1	0·84	1·40	52
Tomato bushy stunt	20·4	27·6	24·5	27·6	55·2	1·13	1·35	52
Turnip yellow mosaic	38·0	22·6	22·2	17·2	39·8	1·02	0·45	52
Southern bean mosaic	23·0	25·8	25·3	25·8	51·6	1·02	1·12	52
Potato *X*	22·8	34·5	21·4	21·4	55·9	1·61	0·94	52
Tobacco ringspot	23·2	23·9	28·2	24·7	48·6	0·85	1·06	52
Tomato bushy stunt	20·8	25·7	25·7	27·9	53·6	1·00	1·34	52
Tomato bushy stunt	20·5	25·7	25·5	28·2	53·9	1·01	1·38	52
Tomato bushy stunt	20·4	25·9	25·6	28·1	54·0	1·01	1·38	52
Aucuba mosaic	18·5	29·7	26·4	25·4	55·1	1·12	1·37	52
Rib grass	18·0	29·3	27·0	25·8	55·1	1·08	1·43	52
Reo	22·0	28·0	27·9	22·3	50·3	1·00	1·01	53
Wound tumour	19·1	31·1	31·3	18·6	49·7	0·99	0·97	53
MS2 phage	22·8	25·2	24·9	27·1	52·3	1·01	1·19	54
f2 phage	26·9	22·2	25·1	26·0	48·2	0·88	0·97	55
R 17 phage	24·9	23·1	25·7	26·3	49·4	0·90	1·06	56
MS2 phage	25·5	23·3	24·8	26·4	49·7	0·94	1·04	43
Poliomyelitis	19·5	30·4	24·7	25·4	55·8	1·23	1·30	52
Influenza *A PRB*	24·0	23·1	32·8	20·1	43·2	0·70	0·84	52
Influenza *B LEE*	23·1	23·0	35·6	18·3	41·3	0·65	0·79	52

has been shown in one case to act as an *m*-RNA[19]. On the assumption that some viral RNAs and perhaps all are indeed *m*-RNAs, attention is now directed to their base compositions. Table 2 lists the various viruses and their RNA base compositions. The properties of viral RNAs can be summarized as follows:

(1) *Percentage of A + G or the purine content of viral RNAs.* In all cases except for turnip yellow mosaic virus and the influenza viruses, the purine content is 50 ± 1 per cent or significantly higher than 50 per cent. Many cases exist in which the purine content is 55 per cent. In the three cases in which the purine content is less than 50 per cent, the molar content of A + G is about 40 per cent.

(2) *The A/U ratio.* About two-thirds of the analyses show that the A/U ratio is equal to or greater than 1·0. Of the one-third in which the A/U ratio is less than 1·0, some have a ratio as low as 0·65.

(3) *The G/C ratio.* In all cases except for turnip yellow mosaic virus and the influenza viruses, the G/C ratio is equal to or greater than 1·0. An extremely low G/C ratio is seen with turnip yellow mosaic virus RNA.

Before these facts are related to present knowledge of *m*-RNA compositions, a pertinent question can be asked. What type of information is present in *m*-RNA ? According to present coding theories, the codon for each amino-acid and the sequence of amino-acids in the polypeptide product are determined by the sequence of nucleotides in the *m*-RNA (ref. 25). Since the *m*-RNA interacts with ribosomes during protein synthesis in an ordered manner, a recognition or starting and stopping sites for translation may be encoded into the ends of the polynucleotide[24]. Furthermore, evidence has been presented for polycistronic *m*-RNA[26–29], that is, an *m*-RNA which codes for more than one polypeptide chain. If this is indeed the case, there must exist a mechanism which allows ribosomes to recognize the end and beginnings of the cistrons. This mechanism may allow ribosomes to separate from the *m*-RNA or it may allow the finished polypeptide chain for each cistron to separate from the ribosome which continues in the translation process[30]. This mechanism may involve specific nucleotide sequences. Therefore the total base composition of an *m*-RNA population would reflect the composition of the codons for the amino-acids in the proteins and other sequences of nucleotides which in some way 'regulate' the translation process.

Two general compositional properties of *m*-RNA are significant: (i) *m*-RNAs appear to be either purine rich, or have equal contents of purines and pyrimidines; and (ii) the A/U and G/C ratios are higher than 1·0 except in one case. For viral RNAs, (*a*) the purine content is higher than the pyrimidine-level in most cases examined; (*b*) the G/C ratio is equal to or greater than 1·0 in all cases examined except three; (*c*) for the A/U ratio, only two-thirds of the virus RNAs had a ratio equal to or greater than 1·0; this is significantly different from the results with *m*-RNAs.

Is there any significance or relationship of these data to other facts which are known about the information transfer

process in living systems ? From recent results obtained by a variety of techniques and by many investigators, certain information has appeared which seems to have relationship to the data presented in Tables 1 and 2.

Several groups have reported that only one strand of the double-stranded DNA is transcribed. Direct evidence has been obtained with phage systems in which phage specific *m*-RNA hybridized with only one of the two strands of phage DNA[31-34]. This information-carrying strand has been the pyrimidine-rich DNA strand in all cases reported. Thus the phage *m*-RNA are rich in purine. This suggests then that the purine-rich *m*-RNA found in non-phage systems may also be significant. It implies that information carrying genetic sites may in all cases be rich in pyrimidine.

The high purine content of the *m*-RNAs examined so far suggests that adenylate and guanylate may be encoded into sequences of nucleotides regulating translation. Recent reports appear to support this contention. In investigations with aminoacyl-transfer-RNA binding directed by trinucleotides[35,36] and polynucleotides[37], the sequences AGA and UAA did not support binding of any of the aminoacyl-transfer RNAs tested. The presence of two adenylates in both cases seems significant in view of the high adenylate content of natural *m*-RNAs. From a theoretical point of view (triplet code) an *m*-RNA with a $1{\cdot}2 \times 10^6$ mol. wt. can code for six polypeptides of $2{\cdot}0 \times 10^4$ mol. wt. If 3 per cent of the nucleotides in this polycistronic *m*-RNA were involved in regulatory functions and two-thirds of these nucleotides were adenylate, the distribution of bases would show that adenylate is present at a molar level 2 per cent higher than that of the other bases. This calculation is based on equal distribution of the remaining 98 per cent of the bases. The percentage of nucleotides involved in regulation may be proportionately even higher, since it was demonstrated recently that a naturally occurring *m*-RNA with 36 nucleotides coded for the *in vitro* synthesis of a polypeptide containing ten amino-acids[38]. In addition, both terminal nucleotides of this natural *m*-RNA were adenylates. The low purine content of HeLa cell *m*-RNA may be significant, since in cancerous cells one might expect some deficiency in the regulatory mechanism. This deficiency is perhaps in the information-carrying DNA strand in which pyrimidine sequences are altered or decreased.

It would be of interest to learn whether triplets with two guanylates are inactive in binding. The triplet GGU was shown to bind glycine[35], but the results with the other possible sequences have as yet to be reported. As the G/C ratio is usually greater than 1·0 in *m*-RNAs and the genetic code appears to be universal[25], it is a possibility that in DNAs with high %GC, the G containing regulating sequences may be more abundant. In this respect, *P. aeruginosa* has an A/U ratio of 1·0 but a G/C ratio of greater than 1·0. In DNAs with high %AT, the A-containing regulating sequences may be more abundant.

The presence of regulating sequences containing A or G can be masked in many ways including high frequency of amino-acid codons which are low in A and G, large *m*-RNAs with few cistrons, or large cistrons with few regulating sequences. These and other reasons may be involved in masking the regulating purine content of RNA from RNA-containing viruses. Although degeneracy in coding probably alters the composition of the *m*-RNAs in different species, the recent work from Nirenberg's laboratory[35,36,58] suggests that all four bases are involved with equal frequency. Furthermore, the purine relationship is seen in organisms with high and low %GC in their DNAs.

Although the proposal of regulatory sequences containing purines is hypothetical, the fact remains that well-characterized *m*-RNAs do have compositional properties which appear to fit a pattern. It is of added interest that this pattern holds true for bulk RNA compositions (ribosomal and transfer (RNAs) as tabulated by Belozersky and Spirin[39]. Recent advances on protein-synthesizing systems[40,41], on the use of natural RNA templates[38], and on RNA sequence analysis[42] have indicated that direct experimental evidence should emerge soon to test this hypothesis.

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CHANGES IN THE *t*-RNA METHYLATING ENZYMES DURING INSECT METAMORPHOSIS

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A NUMBER of enzymes the function of which is the specific methylation of nucleic acids is now known to exist[1]. While considerable information has been gathered about the mechanism of action and specificities of these enzymes, very little is known about the biological function achieved by the methylation of nucleic acids. That the methylation of DNA confers an individuality in one instance has been demonstrated by Arber[2], who found that host range modifications in λ-bacteriophage may be introduced by methylation.

For the enzymes which methylate *t*-RNA, the *t*-RNA methylases, no biological function has been found as yet. It has been demonstrated in our laboratory, however, that the RNA methylases can be profoundly altered in bacterial cells on phage infection or induction[3].

Both the RNA and DNA methylases are species specific[1,4,5]. We have speculated whether the species specificity of these enzymes may not point to their involvement in differentiation, a process requiring the greatest species specificity[1]. We have now tested this hypothesis in an insect during its cycle of metamorphosis and wish to report that alterations occur in the *t*-RNA methylases not only at the transition from larva to pupa but also throughout the duration of the pupal stage right up to the emergence of the imago.

Collection of pupae and their development into adult beetles. *Tenebrio molitor* larvae were purchased from a local supply house and were maintained in open polyethylene trays on a diet of oatmeal, wheat bran and sliced carrots.

Prior to transformation into pupae, the larvae were found to have passed into an immobile phase close to the surface of the food bed. These were transferred to open glass trays and left at room temperature. Pupae were gathered from these trays daily at the same time. Pupae collected on a particular day were formed sometime during the preceding 24 h and were referred to as '1-day-old'. They were incubated at 28° in a humid atmosphere. About 50 per cent of these pupae developed into adult beetles on the seventh day. The process was nearly complete on the eighth day. Pupae ranging in age from one to seven days were picked up from these stocks and were used in the manner described below. The larvae were also collected separately and used similarly for assay of RNA methylases. (Methyl-^{14}C)-*s*-adenosyl-L-methionine with a specific activity of 29·9 mc./mmole was purchased from New England Nuclear Corporation and diluted with non-radioactive *s*-adenosyl-L-methionine to give a concentration of 10 μc. per 0·5 μmole per ml.

Methyl deficient *t*-RNA was prepared by procedures previously published. Protein was estimated by the method of Lowry *et al.*[6].

Preparation of enzyme extracts. Larvae or pupae were crushed and ground in a pre-cooled mortar with three volumes of a solution containing sucrose (0·25 M), magnesium chloride (0·001 M), and 2-mercaptoethanol (0·005 M). The homogenate was transferred into a Potter–Elvehjem type homogenizer. The mortar was washed with an equal volume of the extracting solution and the washings were added to the homogenate. The mixture was next homogenized for one minute with a motor-driven 'Teflon' pestle at 3,500 r.p.m. The homogenate was cooled for 5–10 min in an ice bath and the homogenization was repeated for another minute. The homogenate was then filtered through cheese cloth and the filtrate centrifuged for 20 min at 27,500*g* in a refrigerated centrifuge. The faintly yellow-coloured supernatant was filtered to free it from membrane-like material floating on the surface. This filtrate, which contained 8–12 mg protein per ml. (depending on the stage of development of the pupae), was used as the source of enzymes for the methylation studies reported below. The enzyme extract was prepared fresh daily and was used immediately thereafter. All the operations connected with the preparation of the enzyme extracts were carried out in the cold (2°–4°) unless otherwise stated.

Assay of methylase activity. Methylase activity of the extracts was assayed by measuring the incorporation of ^{14}C-methyl from (methyl-^{14}C)-*s*-adenosyl-L-methionine into methyl-deficient *E. coli* *t*-RNA. The reaction mixture contained 50 μmoles *tris*-HCl buffer, *p*H 8·2; 5 μmoles $MgCl_2$; 5 μmoles 2-mercaptoethanol; 20 μmoles of (methyl-^{14}C)-*s*-adenosyl-L-methionine (20 mc./mmole); 0·15 mg methyl-deficient *E. coli* *t*-RNA, water and the appropriate amount of enzyme extract in a final volume of 1·0 ml. Enzyme extract was added to the reaction mixture which had been pre-incubated at 37°. After 30 min, the reaction was terminated by the addition of 1·0 ml. of cold 10 per cent TCA followed by 3 ml. of 5 per cent TCA. The contents were cooled and centrifuged. The precipitate containing the protein and RNA was thoroughly agitated with 5 ml. of 5 per cent TCA, centrifuged as before and the supernatant discarded. The precipitate was similarly washed twice more with 5 ml. of TCA. Finally, the precipitate was dissolved in 1 ml. of 0·2 N ammonium hydroxide solution and was transferred quantitatively into stainless steel planchettes. The solution was evaporated to dryness under an infra-red lamp and the radioactivity measured in a Nuclear Chicago gas-flow low-background counter. Control experiments were simultaneously carried out in which RNA was omitted. The difference in radioactivity between the experimental and control tubes provided a measure of methyl groups incorporated into methyl-deficient *t*-RNA.

One unit of enzyme is defined as that amount which catalyses the incorporation of 1 μμmole CH_3 into *t*-RNA in 30 min under the foregoing conditions. Specific activity is defined as units of activity per mg of protein.

The amount of enzyme taken, 0·3–0·6 ml., was such that the methyl groups incorporated into *t*-RNA in 30 min did not exceed 40 μμmoles. Within this range, the reaction was proportional to concentration of enzyme as well as to the time of reaction.

Extent of methylation. This was measured as in the assay of activity by incubating the reaction mixture for 4 h with 0·6 ml. of enzyme extract. No appreciable increase in methylation was noticed by incubating for a longer period.

In Fig. 1 the results of examination of the rate of incorporation of methyl groups into a methyl deficient *E. coli* *t*-RNA by extracts derived from larvae in their terminal stage and from pupae at various stages are presented. It is readily apparent that large changes occur in the activity of the *t*-RNA methylases almost daily within the pupae. The experiments were performed

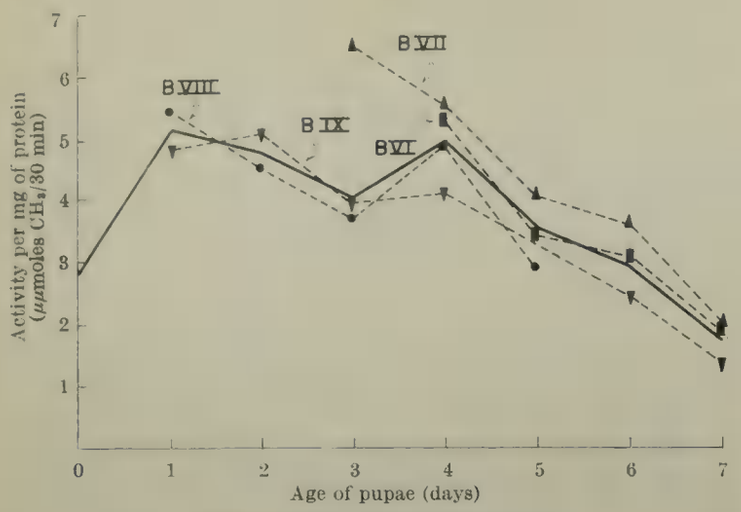

Fig. 1. Activity of *t*-RNA methylases

on different batches of larvae and pupae over a period of several months, yet the pattern of enzyme activity is reproducible. A minimal activity on the third day is followed by a maximum on the fourth. On the seventh day, just prior to the emergence of the beetle, enzyme activity is at its lowest.

Another measurable parameter of the *t*-RNA methylases is the extent of methylation of the substrate, that is, the total number of methyl groups introduced by the enzymes in infinite time. Such determinations are presented in Fig. 2. Again large differences in total enzyme activity are apparent. For example, extracts from a 1-day-old pupa can seek out a little more than twice as many sites for methylation in *t*-RNA as extracts from a 7-day-old pupa. There is excellent concordance between the rates and total enzyme activity of the organisms in the same stage of their cycle of metamorphosis. Only in one batch of pupae (*B* VII) was there a 1-day discrepancy in reaching maximal activity in mid-pupa stage. The deviation from the norm by 1 day in this batch underlines the remarkable synchrony of the other batches of larvae and pupae.

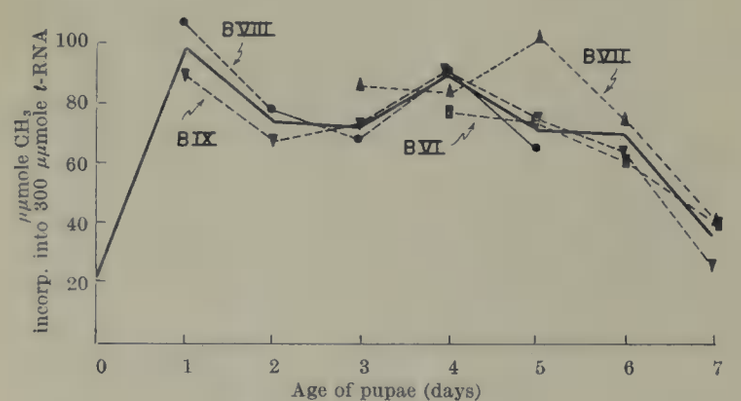

Fig. 2. Extent of methylation

It is difficult to assess the biological meaning of these changes in enzyme activity. Interpretations are hindered by our lack of knowledge of the specific biological function of the methyl groups in *t*-RNA. Some regulatory function in protein synthesis for *t*-RNA has been suggested by Ames and Hartman[7]. Methylation of the *t*-RNAs is one method of altering these molecules for changing functions. If such should eventually prove to be the case, then the fluctuations in the *t*-RNA methylases during phases of metamorphosis will assume a new meaning and a search for the new proteins the syntheses of which are directed by the changing patterns of methylating enzymes could be undertaken.

Between the first and seventh day in the life of a pupa there is approximately a 50 per cent reduction in enzyme activity, whether it is measured as a rate or extent of methylation. The natural question posed by this finding is the cause of the reduction: it could be due to some enzyme inhibitor, or the findings could also be accounted for by the appearance of a new nuclease which essentially eliminates all or part of the substrate. In Table 1 the results of experiments designed to probe these possibilities are presented. Various amounts of enzymes from 1-day-old and 7-day-old pupae were admixed and comparisons were made with respect to both rates and extents of the methylating potencies of the extracts. The addition of small amounts of the extracts of 7-day-old pupae which themselves have low activity does not seem to diminish the levels of activity of the extracts from 1-day-old pupae. However, on the addition of larger volumes of the extract from the 7-day-old pupae, the activity, especially in terms of the extent of methylation, does become diminished. It appears as if a certain concentration of some inhibitor must be reached before it becomes effective in interfering with methylation.

Table 1. Activity of Methylases and Extent of Methylation. Mixing Experiments with Extracts of 1-day-old and 7-day-old Pupae

Exp. 1				Exp. 2			
1-day-old pupal extract (ml.)	7-day-old pupal extract (ml.)	Activity	Extent	1-day-old pupal extract (ml.)	7-day-old pupal extract (ml.)	Activity	Extent
0·3		14·5		0·3		11·9	
0·6		57·2	89·5	0·6		34·1	95·5
	0·3	8·5			0·3	7·1	
	0·6	15·2	55·4		0·6	16·5	65·2
0·3	0·3	26·9		0·3	0·3	24·1	
0·6	0·3	40·4	103·4	0·6	0·3	36·6	80·5
0·3	0·6	31·2		0·3	0·6	36·0	
0·6	0·6	46·6	42·0	0·6	0·6	43·6	66·7

Activity is expressed as μμmoles of methyl groups incorporated into 0·15 mg of *t*-RNA in 30 min. Extent is expressed as μμmoles of methyl groups incorporated in 4 h. In these experiments the total volume of the reaction mixtures was 1·5 ml.

Nuclease activity in extracts of the pupae was also examined as a possible cause of the alterations observed here. Even after incubation for 4 h with these extracts, very little, if any, nuclease activity could be detected. Therefore, the reduced capacity for methylation in the extracts of the 7-day-old pupa cannot be due to the diminution of the substrate but rather to some inherent attribute of the methylases, the nature of which is obscure at present.

A rather potent nuclease activity which cleaved *t*-RNA was detected occasionally in larvae. The appearance of this nuclease activity was correlated with the conditions of the housing of the larvae. The nuclease appeared and reached a maximum within a period of two weeks in larvae which were kept in a relatively dry atmosphere. Under these conditions, the process of pupation was slowed down and ultimately ceased altogether, resulting in the death of large numbers of larvae.

The almost daily alterations in the levels of the RNA methylases during the pupal stage are highly suggestive of some basic function. This is a period of extensive differentiation aimed at the production of the new tissues and organs of the imago. The *t*-RNA methylases alter the structure of one of the cardinal components of the protein synthesizing machinery of the cell. We have also observed changes in the *t*-RNA methylases in an entirely different biological system during upheavals in its metabolic pattern. Either in bacteria infected by *T*2 bacteriophage or in lysogenic bacteria after induction of the latent phage by ultra-violet irradiation, major alterations in the enzyme-levels as well as in the patterns of methylation have been noticed[3]. In other words, in both instances, as a result of changes in the methylating enzymes, alterations must occur in the relative amounts of the methylated bases in the *t*-RNA of the host cell. It is difficult to imagine that bacteriophage carry information within their highly limited volume to make alterations in one of the macromolecules of the host without some purpose. We must therefore search for some regulatory mechanism at the level of *t*-RNA which is triggered by methylation.

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INFLUENCE OF HETEROLOGOUS HISTONE ON THE SYNTHESIS OF CELLULAR AND VIRAL RIBONUCLEIC ACID AND PRODUCTION OF HAEMAGGLUTININ

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IT has been shown that histones inhibit cellular RNA synthesis both *in vivo* and *in vitro*[1–4]. This effect seems to be connected with the histone-induced blockage of DNA as a template. On the other hand, a protein of a histone type was found to be produced in a cell in the first hours after its infection with a virus. This protein inhibits the cellular RNA synthesis before the onset of the viral RNA synthesis[5]. The synthesis of histone in virus-infected cells increased during the first hours after infection[6]. These data suggest that treatment of the infected cells with histone may stimulate the viral RNA synthesis.

In the present work the action of histone on the synthesis of cellular and viral RNA as well as on haemagglutinin production and infectious properties of virus has been studied.

The experiments were carried out on monolayer cultures of chicken fibroblasts. Fowl plague virus (Weybridge strain) was obtained as allantoic fluid of eleven-day-old chicken embryos. In some experiments the virus was concentrated by one cycle of adsorption and elution on chicken erythrocytes. The multiplicity of infection was about 10 and 100 ID_{50} per cell, respectively. The period of adsorption was 30 min at 4° C; the same volume of balanced salt solution was added to the control cells. After washing, the histone in balanced salt solution was added, and the cells were incubated at 37° C; this moment was considered as zero time of the experiment. ^{14}C-cytidine was added to the medium every hour or every half-hour after infection. The monolayers were washed, cells taken off the glass, pipetted, collected on 'Millipore' filters, treated twice with cold 5 per cent perchloric acid, twice with cold 96 per cent ethanol, and dried. After counting, the 'Millipore' filters were dissolved in acetone, RNA was hydrolysed by 1·7 M $HClO_4$ for 18 h at 4° C and the radioactivity was counted per μg RNA.

The total histone was isolated from the calf thymus by the method of Hnilica and Hupka[7] in the Institute of Medical and Biological Chemistry, U.S.S.R. Academy of Medical Sciences.

In some experiments cells were incubated in the presence of 5-fluorodeoxyuridine (FUDR), 10^{-6} mole/ml. being added at zero time.

As can be seen in Fig. 1, histone inhibited ^{14}C-cytidine incorporation in the acid-insoluble fraction during the first 2–3 h. The same results were obtained in the presence of FUDR. Hence this effect is connected with the inhibition of RNA synthesis.

The inhibition of incorporation (varying from 12 to 60 per cent of the control-level) occurred in the first 2 h after infection with the allantoic virus. The incorporation was maximal 4–5 h after infection and reached about 130 per cent of the control. When concentrated virus was used

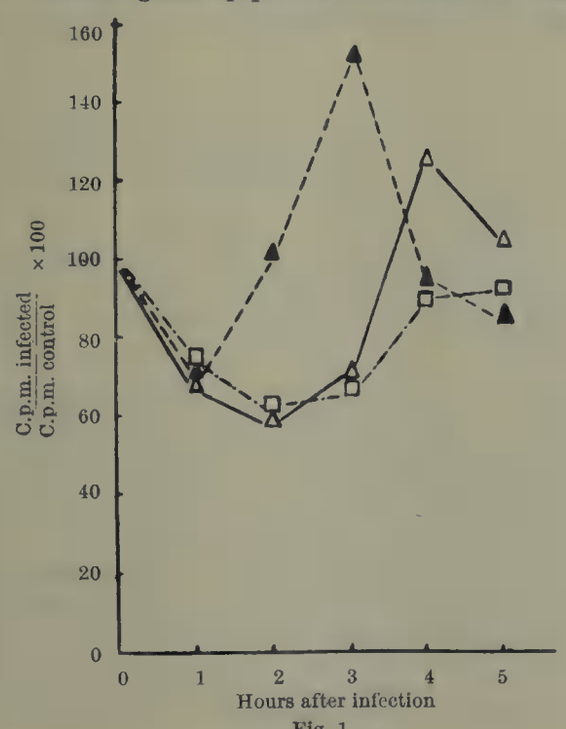

Fig. 1

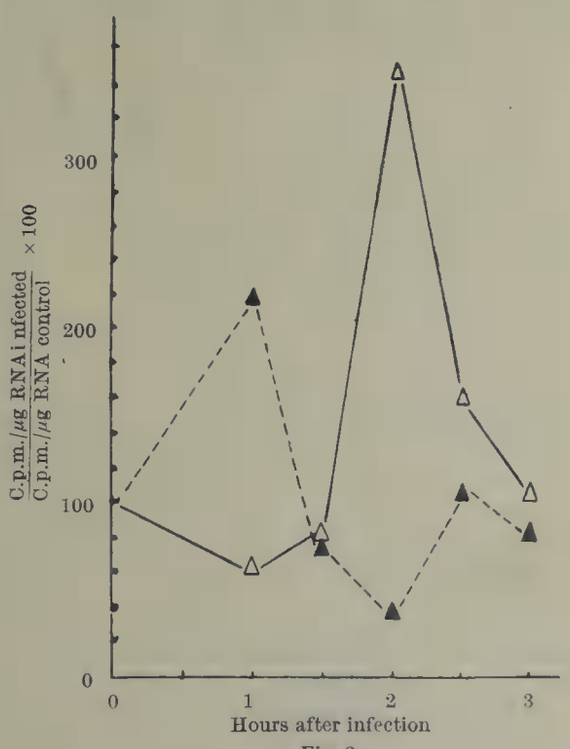

Fig. 2

Figs. 1 and 2. Rate of ^{14}C-cytidine incorporation in acid-insoluble fraction of chicken fibroblasts. Radioactivity of histone-treated non-infected (□) and untreated infected (△) cells as percentage of the control; radioactivity of histone-treated infected cells (▲) as per cent of radioactivity of histone-treated non-infected cells. (1) Multiplicity of infection 10 ID_{50} per cell; each point is the average of 4 sets of experiments with 100 μg/ml. of histone. 0·07 μc./ml. of ^{14}C-cytidine (specific activity 2·5 μc. per μmole) were added in 2–3 flasks 0, 1, 2, 3 or 4 h after infection and left for 1 h. (2) Multiplicity of infection 100 ID_{50} per cell; one typical experiment with 150 μg/ml. histone. 0·07 μc./ml. ^{14}C-cytidine were added 0, 0·5, 1, 1·5, 2, 2·5 or 3 h after infection and left for 0·5 h

the minimal and maximal incorporation was found after 1 h and 2–2·5 h, respectively. Similar results with infected cells were observed in the presence of FUDR (Figs. 1 and 2). The inhibition of the incorporation seems to be caused by the formation of virus-induced repressor of cellular RNA synthesis while the stimulation of the incorporation represents the viral RNA synthesis[8].

In the experiments with histone treatment the maximum of incorporation after infection with non-concentrated virus was higher than in the infected control, the minimum and maximum of incorporation were observed at 1·25 ± 0·3 h and 2·8 ± 0·43 h, respectively; whereas in the experiments without histone these intervals were 2·25 ± 0·3 h and 4·4 ± 0·28 h, respectively. The maximum of the incorporation in histone-treated cells infected with concentrated virus was lower than in infected controls and occurred 1 h earlier (Figs. 1 and 2). Thus, histone treatment brought about inhibition of cellular RNA synthesis and earlier synthesis of viral RNA. It may be supposed that heterologous histone acts similarly to virus-induced repressor. Their combined action inhibits cellular synthesis of RNA earlier and, probably due to this, earlier synthesis of the viral RNA occurs. There would seem to be a relationship between the time of the cellular RNA synthesis inhibition and that of the viral RNA reproduction.

The haemagglutinin titres and ID_{50} in histone-treated cells were lower as compared with infected controls (Table 1).

Table 1. Histone Action on Haemagglutinin and ID_{50} Titres at Different Times after Infection

	Hours after infection						
	2	4	6		8	12	
Experimental series	HA	HA	HA	ID_{50}	HA	HA	ID_{50}
Control cells	0	16	32	$10^{7.5}$	64	128	$10^{7.5}$
Histone-treated cells	0	2	4	$10^{7.0}$	8	16	$10^{7.0}$

Cells were infected with 10 ID_{50} per cell, thoroughly washed, and 100 μg/ml. of histone were added at 0 time.

The cells were disrupted 12 h after infection by quick-freezing and thawing, haemagglutinin titres were determined in supernatant fluid with a 0·5 per cent suspension of chicken erythrocytes, ID_{50} titrated in chicken embryo pool and expressed as haemagglutinin units/ml. and ID_{50}/ml.

It is of interest to note that probably there is a comparatively narrow time-interval over which the addition of histone to the medium can influence haemagglutinin production. After the treatment of cells from 0 to 2 h, haemagglutinin titres were 8–16-fold lower than those in the control; when histone was added 3 or 5 h after infection, haemagglutinin titres were about the same as in the control. In three experiments histone was added

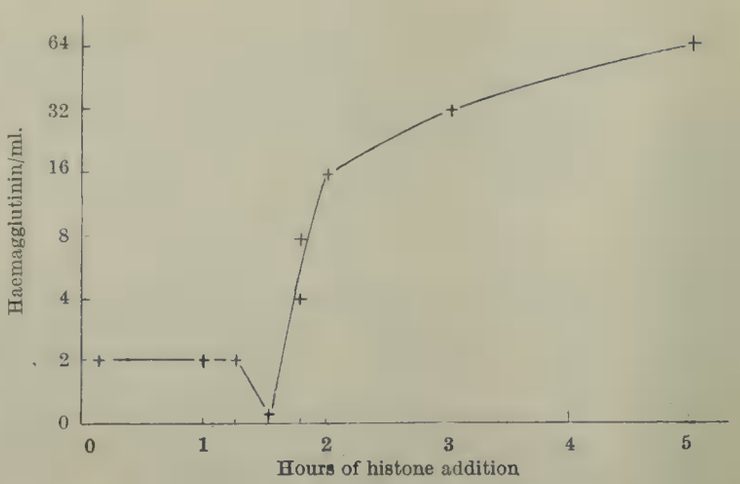

Fig. 3. Haemagglutinin production in relation to the time of histone addition. Each point is the average of 3 experiments. Cells were infected with 10 ID_{50} per cell and thoroughly washed, and 100 μg/ml. histone were added 0, 1, 1·25, 1·5, 1·75, 2, 3 and 5 h after infection. The haemagglutinin titres were determined as described in Table 1

to the medium every 15 min after infection. The most sensitive period to histone action was between 0 and 1·5 h after infection, the haemagglutinin titres were lowest when histone was added 1·5 h after infection (Fig. 3). Haemagglutinin production in this system starts 3 h after infection. Thus the most sensitive period was found much earlier than the process of haemagglutinin production started.

It is not yet known whether the cell genome takes part in haemagglutinin production of myxoviruses. That is why it is difficult to decide whether the effect observed is due to the binding of histone with DNA or viral RNA. Of interest is the fact that actinomycin *D* in low concentration (up to 2 μg/ml.) has the same effect as histone on the synthesis of the viral RNA and haemagglutinin of *PR*8 and Weybridge strains of fowl plague virus[9,10].

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SUPPRESSION OF THE PUPILLARY LIGHT REFLEX IN BINOCULAR RIVALRY AND SACCADIC SUPPRESSION

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In 1948 Bárány and Halldén[1] reported that the pupillary response to a flash presented to the non-dominant eye of a subject experiencing binocular rivalry was smaller than the response to the same flash presented to a dominant eye. We have sought to repeat these experiments using the direct-recording infra-red pupillometer[2] in order to get an objective measure of this suppression.

The subject's head was firmly held in front of the pupillometer by means of a bite board and head rest. The right eye viewed a circular field of about twenty degrees in diameter which was dimly illuminated, and in the centre of which was a small fixation cross at optical infinity. The test flash, when it appeared, was superimposed on this field and had a duration of 10 msec. The fixation target for the left eye was a small dot at optical infinity and in the centre of a uniform field illuminated with a diffuse red light. The stimulus was delivered to the right eye every 3 sec, so that during rivalry flashes were alternately presented during dominance and non-dominance (reported by the subject, who controlled a two-position switch).

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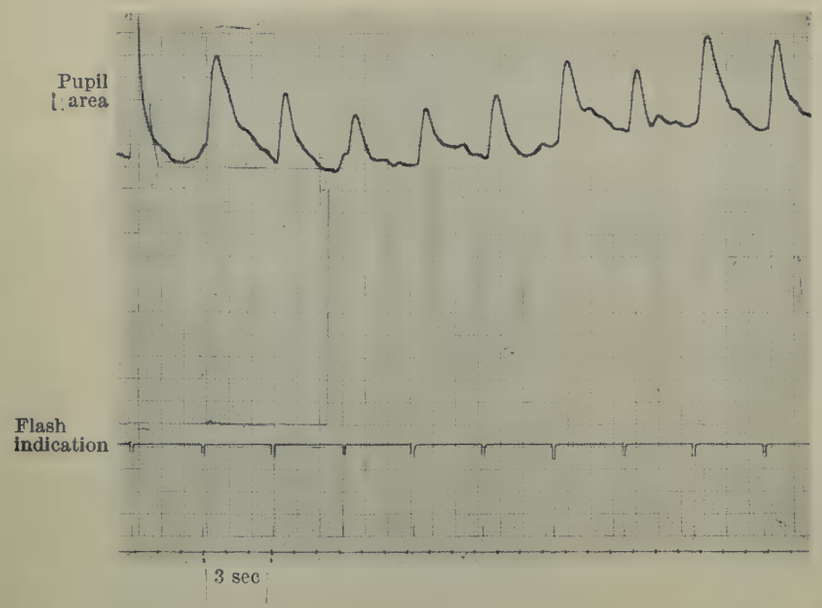
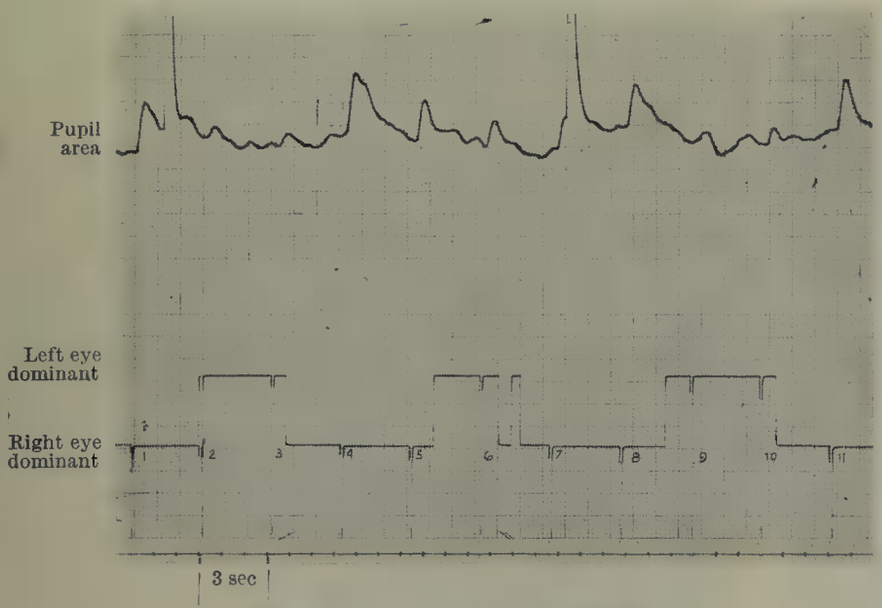


(*a*) Controls with left eye closed



(*b*) Responses during binocular rivalry

Fig. 1. Measurements of the pupillary response during binocular rivalry. *a*, Control responses with the left eye patched; *b*, responses during rivalry. Upper trace shows pupillary responses; broad shifts of base line on lower trace represent subject's state of dominance, narrow pulses indicate stimulus presentations

In Fig. 1*a* the responses to control flashes, taken with the left eye occluded, are shown. Fig. 1*b* shows responses during rivalry. Pupillary responses appear on the upper trace, while the subject's state of dominance (broad shift of the base line) and the occurrence of the stimulus (narrow pulses) are represented on the lower trace. On the whole, the responses during 'right eye dominant' are smaller than control responses, probably because the subject often experienced fusion of the two fields under this condition. There seems to be a significant decrease in the amplitude of the pupillary response when the right eye is suppressed (left eye dominant). These results have been confirmed by Richards[3].

It has been well established that the visual threshold is significantly elevated just before and during a saccadic eye movement[4–13]. This phenomenon has been referred to as 'saccadic suppression'[13]. The experiments described here were designed to determine whether or not the pupillary light reflex is involved in this suppression.

The time required for the completion of a saccadic eye movement is short compared with the latency of the pupillary response. Thus the pupillary response to a flash presented to the moving eye may be measured by the pupillometer well after the eye has come to rest.

The stimulating apparatus (pupillometer) was basically the same as described above. Viewing conditions were monocular with the left eye occluded, and a second fixation cross was placed at the left periphery of the field for the right eye. Eye movement was always from the left peripheral to the central fixation cross, and angular distance of about eight degrees. A signal proportional to eye position was obtained by a previously described method[12]. Test flashes were presented to the subject at various times before, during and after the beginning of a saccadic eye movement, and visual response (seen or unseen), pupillary response, eye movement and stimulus were recorded. A pulse with variable associated delay and constant width (10 msec) was used as the stimulus.

Pupillary responses to test flashes presented during and after the eye movement were averaged on a digital computer (GE 225) equipped with analog-to-digital converter[14]. For these stimuli the eye position signal served as a trigger both for the inputting sequence of the computer and for the presentation of the delayed test flash. The time of presentation of the test flash with respect to the beginning of the eye movement was determined by the delay setting. The subject indicated having seen a given test flash by depressing a push-button which caused a pulse to be registered on the recorder. Thus, visual saccadic suppression was followed on the recorder, while pupillary saccadic suppression was followed on the computer.

Responses to test flashes presented before the eye movement could not be averaged on the computer because: (1) the eye movement could not be used as an initiator; (2) the variability of the latency of eye movement was too great. In this case the subject moved his eye on receiving an auditory stimulus which also served as a trigger for the test flash. This was delayed so little that it occurred during the latency associated with the eye movement. These responses were grouped according to time of test flash presentation and hand-averaged. Saccadic suppression was followed as already described here.

Average responses to test flashes presented during and after the eye movement are shown in Fig. 2. Pupillary responses are shown in arbitrary units. Controls consisted of: curve *A*, responses to the test flash during central fixation, that is, with no eye movement; curve *B*, responses to the test flash during left peripheral fixation (no eye movement); curve *C*, responses to the eye movement with no test flash presentation. In some experiments a small pupillary response was observed in this last control, probably due to a small change in accommodation from one fixation point to the next. The average response for this control was subtracted from all computer-averaged responses (excepting controls) in order to emphasize more clearly trends in the data. Curves *D*–*L* represent average responses to these test flashes (control *C* subtracted out), the time of flash presentation with respect to the beginning of the eye movement appearing to the right of each response. It is clear that the average pupillary response is much diminished for short times of flash presentation. As the time of flash presentation approaches 980 msec the average response amplitude approaches that of the control responses. Fig. 3 shows psychophysical saccadic suppression and pupillary saccadic suppression results from the same experiments where test

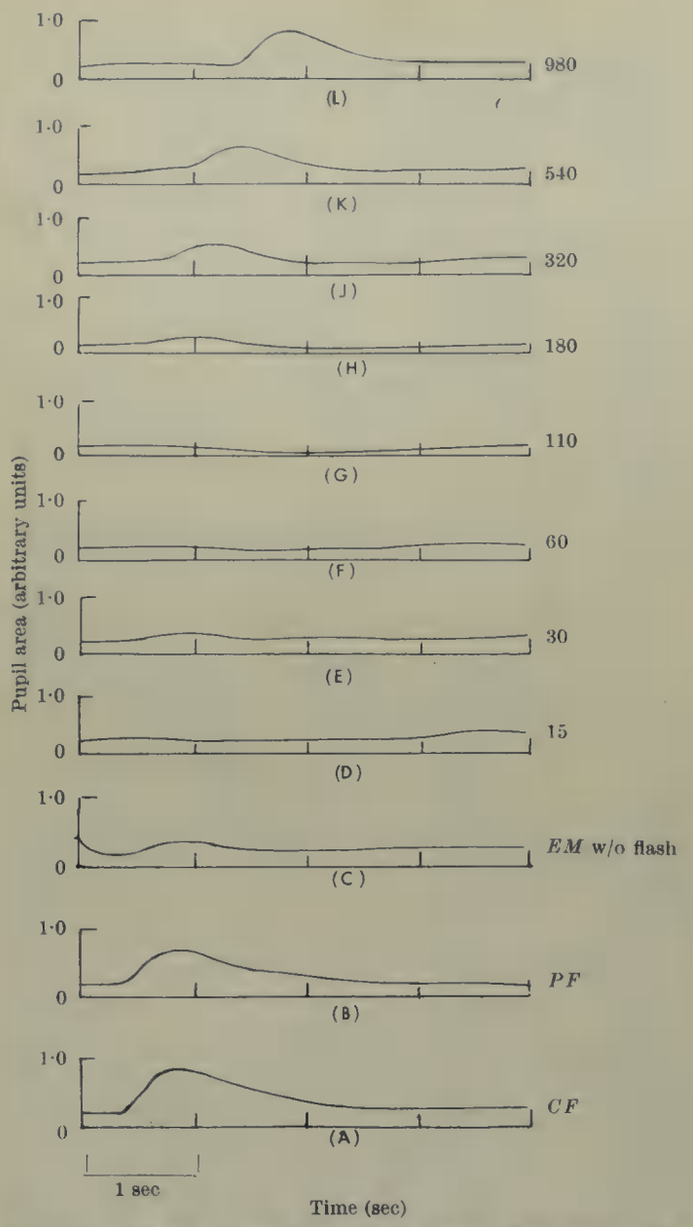


Fig. 2. Pupillary responses to test flashes presented at various times during and after eye movement. Time of presentation in msec appears at right of each average response. Curves *A*, *B* and *C* are controls

flashes were presented before, during and after the eye movement. The visual saccadic suppression observed in this experiment agrees in general with previously published results[7,12]. When saccadic suppression is maximal no test flashes are seen and the average pupillary response is reduced to 10 per cent of the average response to the test flash during central fixation. While it is clear that visual and pupillary saccadic suppression occur concurrently, the time-course of pupillary saccadic suppression seems far more extensive than that of visual saccadic suppression. This experiment has been performed on

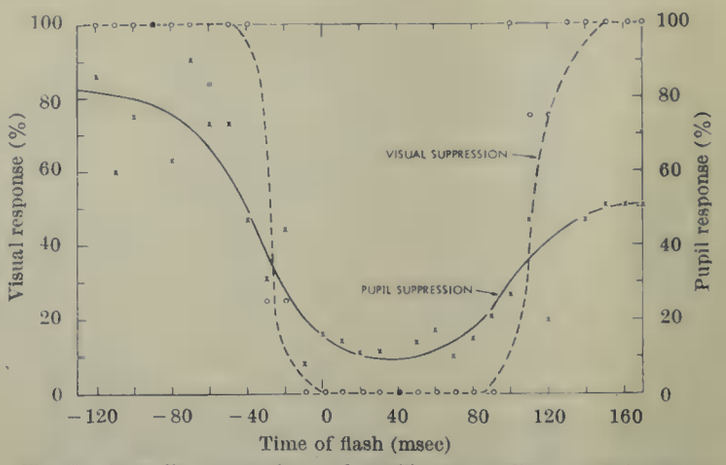


Fig. 3. Saccadic suppression and pupillary saccadic suppression as a function of time of flash presentation with respect to beginning of eye movement ($t = 0$). Visual response represents per cent seen; pupil response represents average amplitude as percentage of amplitude of average response during central fixation

two subjects and the results are quite similar for both.

Much of the pupillary saccadic suppression observed in these experiments occurs before the eye movement, seemingly minimizing the probability that the suppression is caused by an artefact. Possibilities for such artefacts are non-linear addition in the pupillary system[15–17] and illumination of new retinal areas caused by the eye movement.

Based on the results of these experiments we are not able to determine whether the suppression observed in binocular rivalry and that observed in saccadic suppression are generated by the same mechanism. It is hoped that further experiments, both neurophysiological and psychophysical, will further elucidate these as well as many other related phenomena.

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FUNCTIONAL SIGNIFICANCE OF SWEAT GLANDS AND SEBACEOUS GLANDS IN SEALS

By JOHN K. LING

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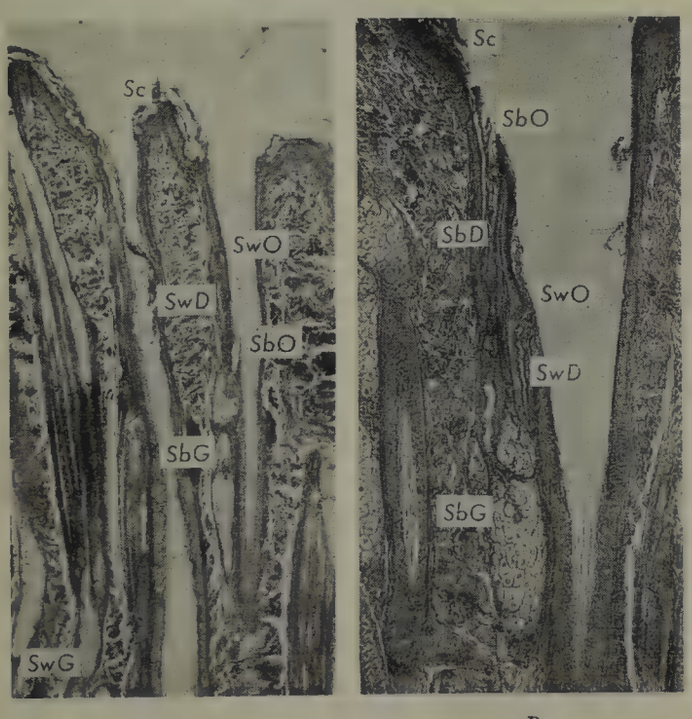
BIOLOGICAL investigations being carried out by Australian National Antarctic Research Expeditions at Macquarie Island (54° 30′ S., 159° E.) include a study of the skin and hair cycle of the southern elephant seal, *Mirounga leonina* (L.)[1]. Skin samples have been collected from five other pinnipeds also inhabiting southern latitudes but from different climatic regions.

It has been regarded as typical among mammals for the apocrine sweat glands to open into the pilary canal above the opening of the sebaceous glands, or even on to the skin surface[2]. In the Phocidae this situation is reversed (Fig. 1), as previously noted in reports on skin from the general body surface[3–5]. Examination of serial sections of skin cut parallel to the skin surface of the

Fig. 1. Section of elephant seal skin from back, cut parallel to hair follicles to show opening, *SbO*, of sebaceous duct, *SbD*, above orifice, *SwO*, of sweat duct, *SwD*. *SbG* = sebaceous gland; *Sc* = stratum corneum. Oil red O and haemalum. × 27·5

body and flippers of three phocid species confirms that the sweat ducts enter the hair canal below the sebaceous gland ducts.

Sweat glands in the Otariidae open into the pilary canal above the sebaceous gland openings over the general body surface (Fig. 2*A*). It is remarkable, however, that the apocrine sweat glands associated with hair groups on the flippers of the Australian fur seal, *Arctocephalus doriferus* Wood Jones, enter the pilary canal by way of a duct opening below the sebaceous gland orifice (Fig. 2*B*). Thus we have opposite arrangements on different parts of the same species of seal. The distally naked areas of skin on the fore and hind flippers have no cutaneous glands, although sections of 'naked' regions on hind flippers of the New Zealand fur seal, *Arctocephalus forsteri* (Lesson), have revealed minute hairs with associated sweat and sebaceous glands. These hairs could not be seen when the outer surface of fixed material was viewed microscopically.



A *B*

Fig. 2. Sections of Australian fur seal skin cut parallel to hair follicles showing relative positions of cutaneous gland openings into pilary canal. *SwG* = sweat gland. Other lettering as for Fig. 1. *A*, Dorsal body surface, × 54. *B*, Dorsal surface of hind flipper. Mallory's triple stain × 64·5

In the true seals (Phocidae) the sweat glands are rather insignificant, though in accurately oriented longitudinal sections their ducts can generally be traced alongside the follicle and seen to open just above the constricted region. Thus the sweat duct passes between the sebaceous gland and the follicle, and the two gland openings are visible one above the other, with the mouth of the sebaceous duct appearing in the form of a delta among the lamellae of the stratum corneum, particularly of the elephant seal. Copious lipid secretion can be demonstrated in frozen sections stained with oil red *O* (Fig. 1).

The sweat glands over the general body surface of the fur seals (Otariidae) are relatively enormous organs extending well below the bases of the hair follicles. Their secretory portions are much coiled and the ducts ascend outside the sebaceous glands to open into the funnel-shaped upper end of the pilary canal above the sebaceous gland opening. On the hairy proximal regions of the flippers of otariids the sweat glands are somewhat reduced and their ducts ascend between the follicle and the sebaceous gland in the same way as on all regions of phocids. In these areas the sebaceous glands are larger and the stratum corneum is thicker and more richly supplied with lipids.

The bilobed sebaceous glands of Phocidae are large compared with the size of the follicle and are situated on its ental side. These glands have unequal lobes in the Otariidae and the larger one enters the ental side of the guard hair follicle, while the smaller and more slender gland opens on the ectal side of the underfur follicles that emerge from a common pilary canal.

With the exception of the harp seal, *Pagophilus groenlandicus* (Erxleben), the relative positions of the cutaneous gland openings on the general body surface are distinct for the two families of seals. Bergersen's[3] meticulous observations on *P. groenlandicus* leave no doubt that the sweat gland orifice in this species lies above the opening of the sebaceous glands. However, in addition to the guard hair, the follicle bundles of the harp seal contain 5–9 subsidiary hairs, quite a high number in comparison with other phocid species.

It has been stated that the naked areas of flippers of all Pinnipedia—including the walrus, *Odobaenus rosmarus* (L.)—are devoid of sweat glands[6]. However, the appearance of liquid droplets on the glabrous palmar surface of the northern fur seal, *Callorhinus ursinus* (L.), after exposure to radiant heat was assumed to indicate that sweating had been induced[5]. Large and abundant sweat glands have been found in microscopic preparations of skin from naked regions of fore and hind flippers of the same species[7].

I recently noticed fine hairs with associated cutaneous glands in histological sections of apparently naked skin from the fore flipper of *C. ursinus* (kindly lent by Dr. V. B. Scheffer). A similar situation obtains in the 'naked' skin of the hind flipper (but not the fore flipper) of *A. forsteri*. No cutaneous appendages have been found in naked skin of the fore or hind flippers of other southern otariids. There were, however, delicate, lipid-rich strands ascending vertically through the stratum corneum on the hairless part of the fore flipper of the Kerguelen fur seal, *Arctocephalus tropicalis gazella* (Peters), but no associated tubules were visible in the dermis. The nature of these structures remains unknown.

The phylogenetic significance of apocrine sweat glands in primates has been discussed recently in terms of the shifting position of the orifice of the sweat duct from the skin surface of the Prosimii to within the pilary canal of man[2]. This latter situation is common for most mammals, an exception being the pig, the glands of which open on to the skin surface[8]. Thus there is no clear relationship between the amount of hair and the position of the sweat gland orifice in mammals described so far.

The morphology of sebaceous and sweat glands in seals may be related to the adaptation of these animals to their aquatic environment. Loss of functional hair in the thermoregulatory sense among the Phocidae and on the flippers of the Otariidae was accompanied (or followed ?) by reduction in size of the sweat glands and a shifting of their excretory pore to a position below that of the sebaceous gland opening. Sebaceous glands are relatively larger in the skin of sparsely haired phocids and on less hairy parts of otariids, and these glands secrete a heavy lipid deposit within the stratum corneum. Although the sweat glands of the walrus—an almost hairless pinniped—are large, they actually atrophy during autumn[9]. The sebaceous glands are relatively small, but the nature of their secretion is not known.

Aoki and Wada[10] have shown that sweat glands on the general body surface of dogs are sensitive to local stimuli such as radiant heat and drugs, but are insensitive to bodily conditions of heat stress. These authors suggested that temperature regulation is the chief function of the apocrine sweat glands of dogs. It is probable that similar local effects of sweat glands are associated with the hair bundles of heavily furred body regions of fur seals and the harp seal.

There is an apparent distinction in relative size and position of the sweat glands and sebaceous glands between the more terrestrially inclined Otariidae and the aquatically adapted Phocidae. This seems to weaken the argument for a biphyletic origin of the Pinnipedia[11]. Sweat glands appear to be functionally important in association with an elaborate pelage where their role is probably the regulation of local skin temperature. With the progressive loss of hair, exemplified by seals of the family Phocidae, the need for water-proofing the skin is met by enlarged lipid-secreting sebaceous glands, thermoregulation being achieved by the thick layer of subcutaneous blubber. It has been suggested that northern fur seals depend on their naked flippers for maintenance of their body temperature[7], but this may not be a general rule in the Otariidae. The density, size and distribution of cutaneous glands, if any, and blood vessels in the naked flippers of Otariidae need to be carefully analysed before their significance can be evaluated.

Further detailed quantitative studies of the skin of pinnipeds and other aquatic mammals are required to elucidate structure and function of the integument and its associated glands. Perhaps the evolutionary path back to the water by these once terrestrial forms will then emerge.

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INTERFEROMETRIC ESTIMATION OF EFFECTS OF TESTOSTERONE AND METHANDROSTENOLONE ON THE DRY MASS OF VARIOUS RAT MUSCLES

By Dr. W. STAÜBLI and Dr. T. OKADA

Research Laboratories, Pharmaceutical Department, Ciba, Ltd., Basle

In animals following castration certain target organs display marked atrophy, which is reflected in a decrease in their wet and dry weights[1]. This loss of weight of hormone-dependent organs can be partly or completely reversed by giving the castrated animals substitution treatment with androgenic[1–3] or anabolic[2] steroid hormones. In the present study we investigated the myotrophic effect of testosterone and methandrostenolone ('Dianabol') on certain skeletal muscles in the castrated rat. The optical path difference of the muscle fibres, as determined with the aid of an interference microscope, was selected as a criterion of the effect exerted by the hormones on the muscles.

Male Sprague–Dawley rats weighing approximately 100 g were used. They were kept on a standardized diet, and offered drinking-water *ad lib.* The rats were divided as follows into four groups, each containing four animals: (1) untreated controls; (2) castrated controls; (3) castrated animals, given one subcutaneous injection of testosterone (10 mg/kg in sesame oil) daily for 14 days, starting on the 15th day after castration; (4) castrated animals, given one subcutaneous injection of methandrostenolone (30 mg/kg in sesame oil) daily for 14 days, starting on the 15th day after castration. The m. levator ani and the m. bulbocavernosus were selected as representative 'sexual muscles'. Since the m. levator ani displays the characteristic features of a 'white' muscle[4], the white portion of the m. psoas was used for comparison purposes.

Immediately after removal, the muscle tissue was fixed in Carnoy's solution, mounted in paraffin, and 4μ sections were prepared. For the purposes of interferometry, the sections, after removal of the paraffin, were covered with glycerol.

The optical path difference of the muscle fibres (φ) was estimated on longitudinal sections of the fibres (Fig. 1) with the aid of a Leitz interference microscope (fluorite objective with a magnification of 50 times, A, 0·85; monochromatic light of $\lambda = 546$ mμ) by measuring the displacement of the interference bands (d/D, Fig. 1) caused by the object. The optical path difference determined in this way is in direct proportion to the dry mass per unit of area[5] ($A1$, $A2$, $A3$, Fig. 1). As the volumes ($V1$, $V2$, $V3$, Fig. 1) of the muscle fibres fluctuated markedly in response to hormonal treatment (Fig. 1), the following volume parameters were determined on photographs of cross-sections stained with haematoxylin-eosin: (1) the number of muscle cell nuclei per unit of area (5 cm × 5 cm); (2) the number of muscle fibres per unit of area; (3) the area of the muscle fibres as estimated by planimetry ($A'1$, $A'2$, $A'3$). Since the thickness of the specimens (t, Fig. 1) was kept constant, the path difference can be regarded as proportional[5] to the concentration of substances ($C = \frac{M}{V}$) in the muscle fibres. The volume V is a prism, the sides of which are marked a, l (length of the muscle fibre), and t (thickness of the section) in Fig. 1.

After the wet weight had been measured, the muscles were dried to constant weight at 110° C for the purpose of estimating their dry weight.

It is known that the areas of the fibre cross-sections in certain target muscles are considerably reduced as a result of castration[6]. Since the muscle fibres in histological

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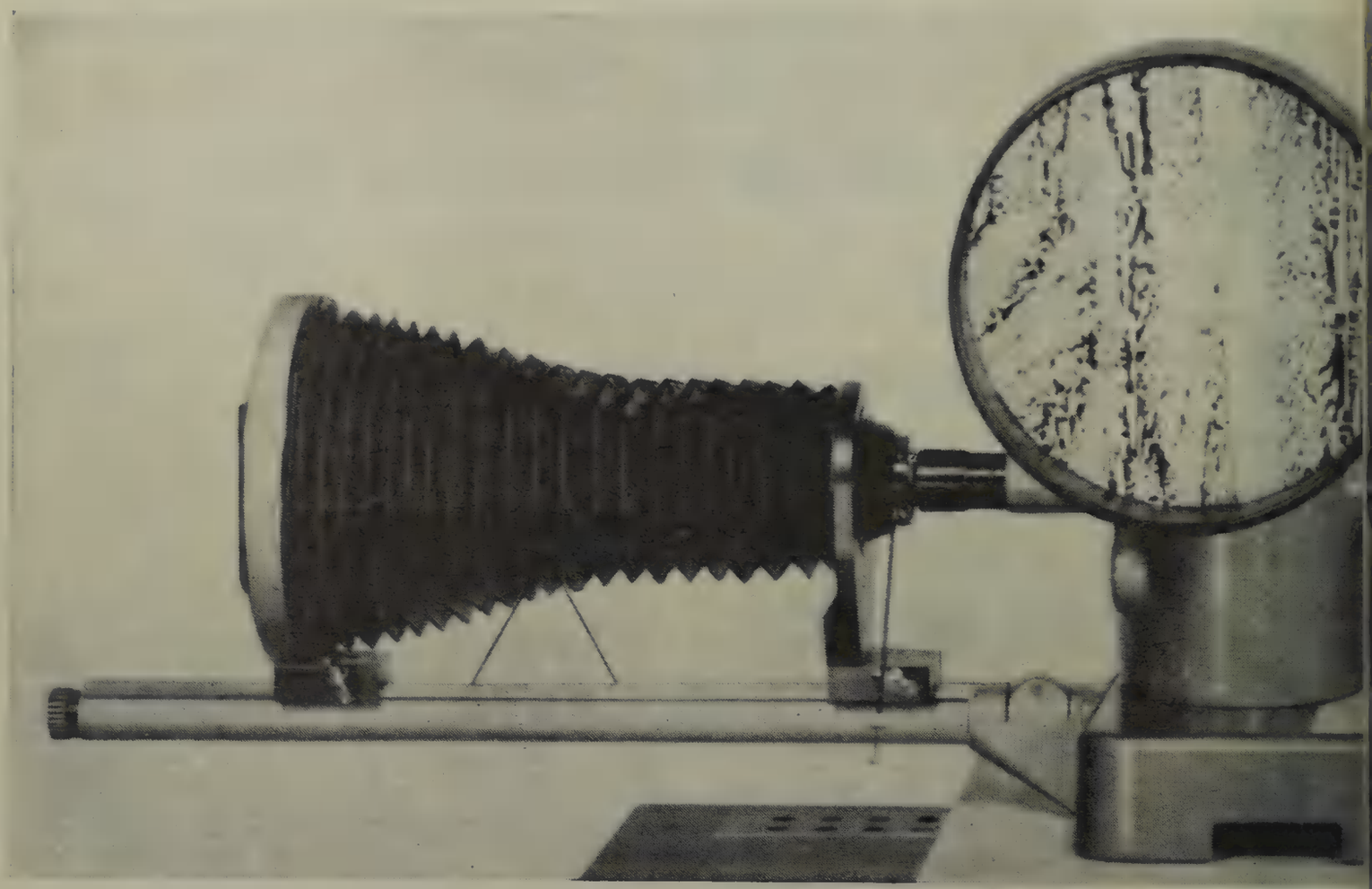

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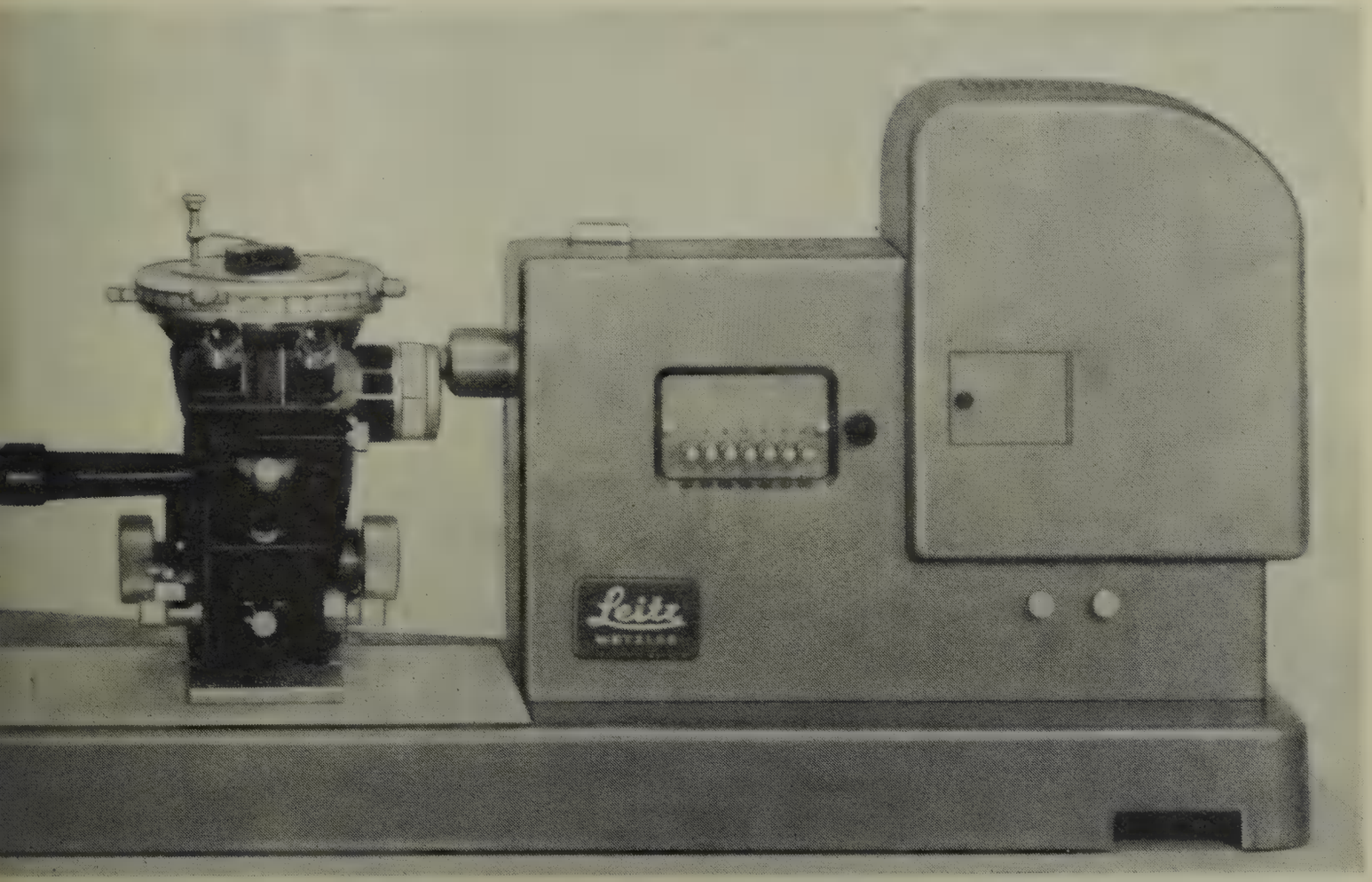

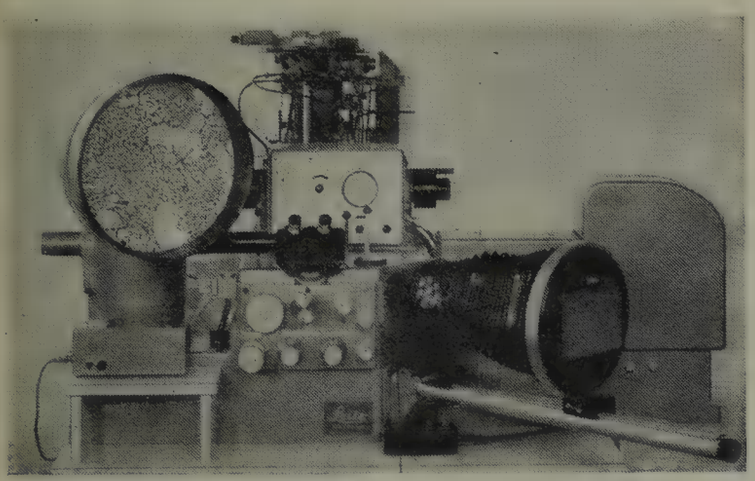

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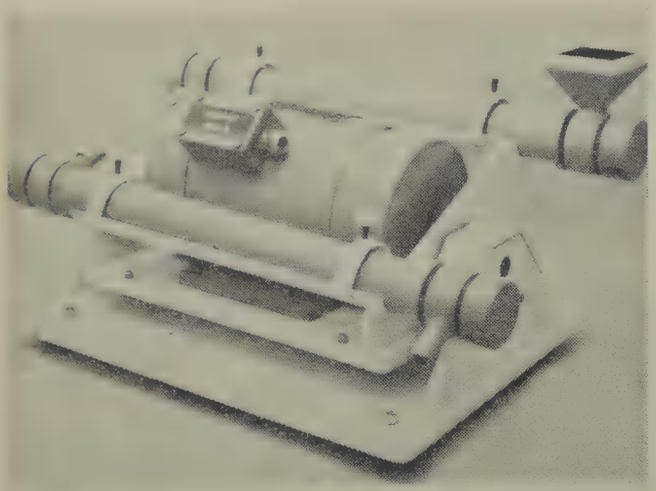

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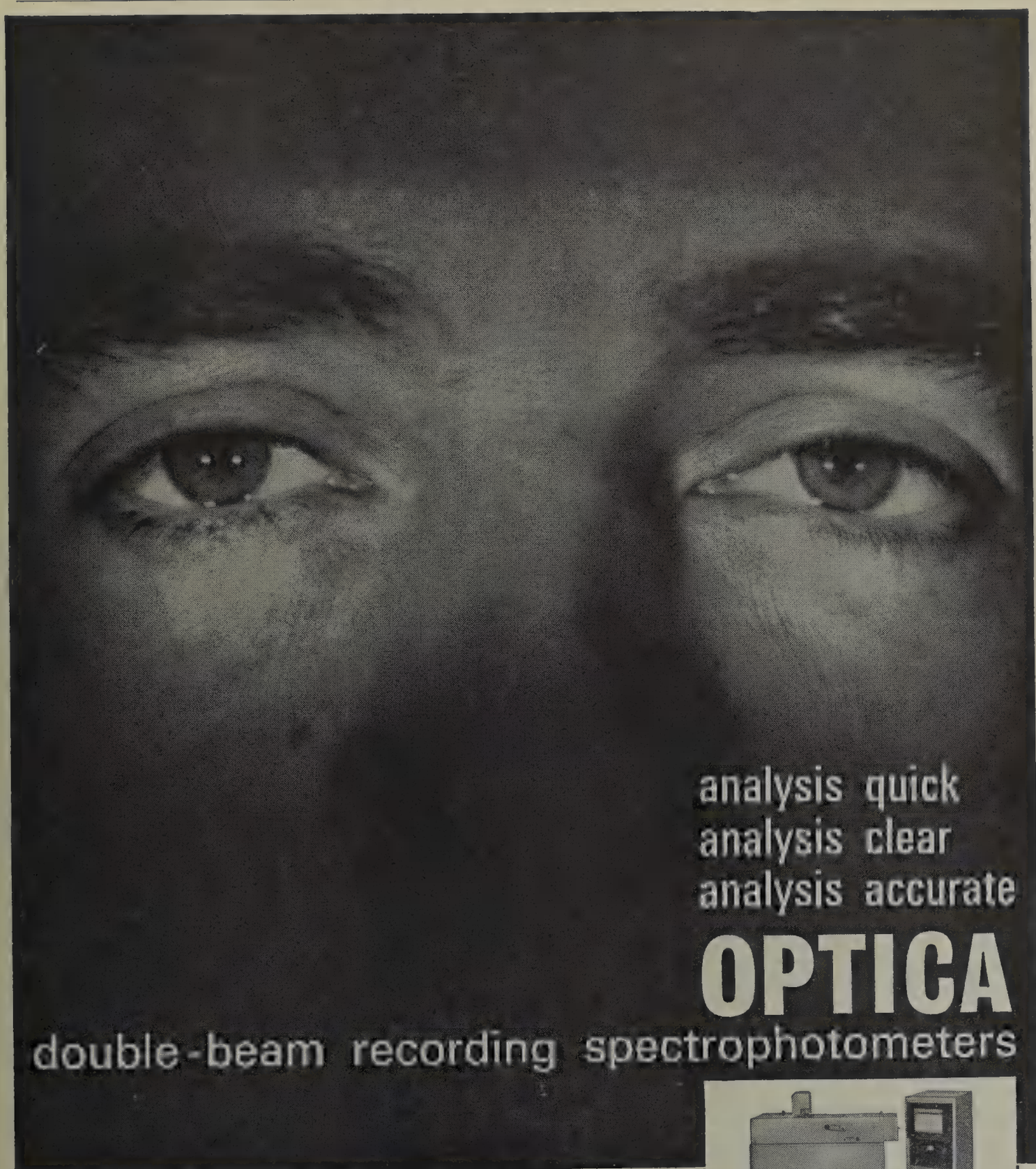

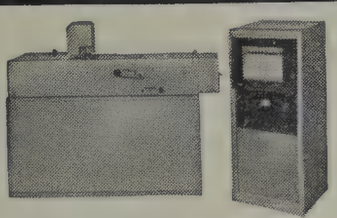

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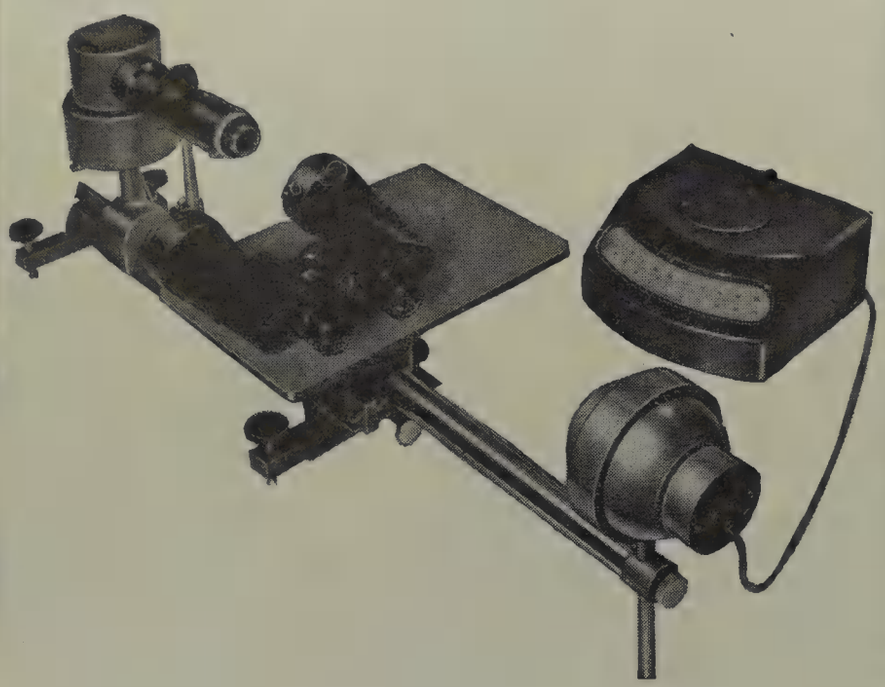

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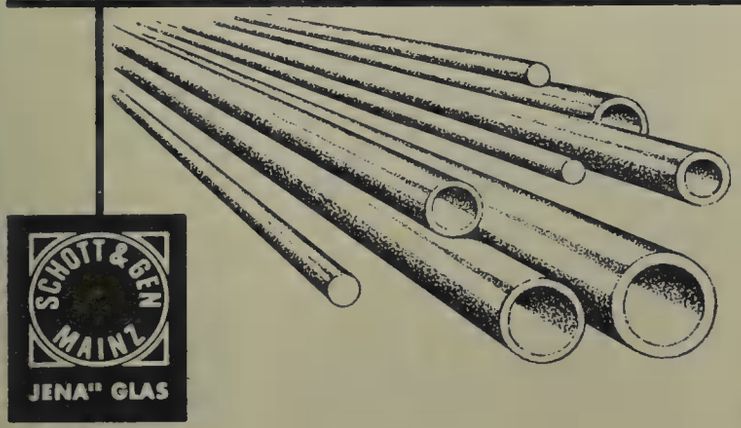

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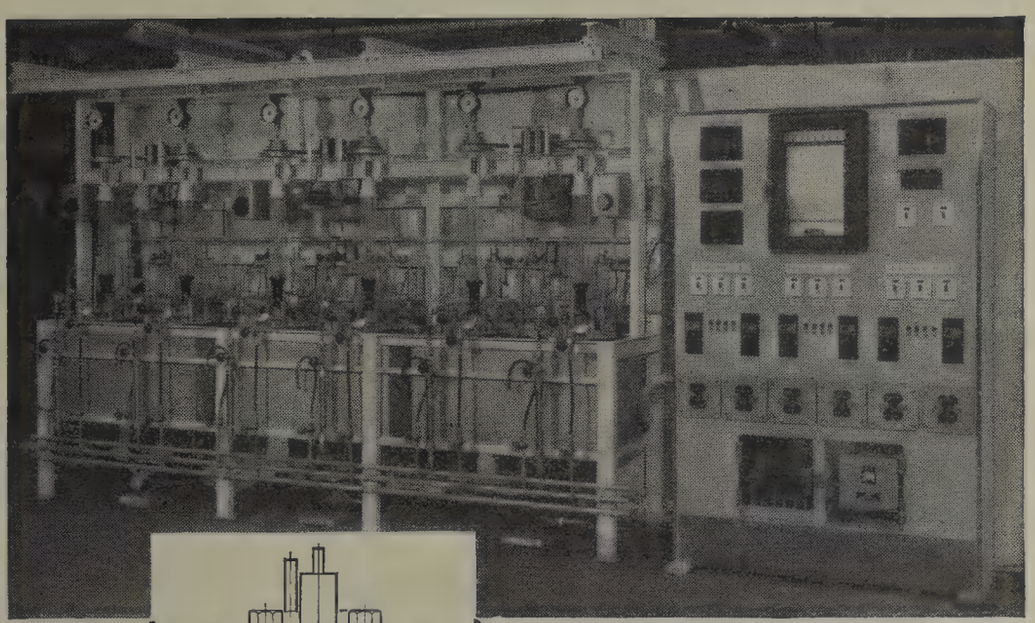

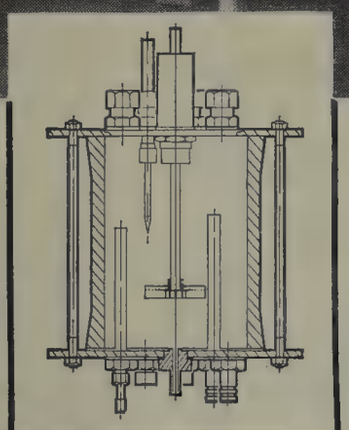

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Table 1. Influence of Hormone Treatment on Volume Parameters of Muscle Fibres

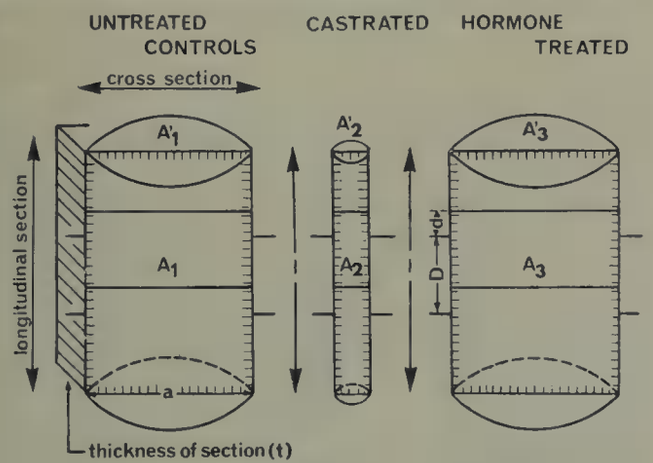
Treatment	M. levator ani						M. bulbocavernosus						M. psoas (white)					
	Nuclei		Fibres		Area of fibres		Nuclei		Fibres		Area of fibres		Nuclei		Fibres		Area of fibres	
	No. (1a)	% (1b)	No. (2a)	% (2b)	Mean (3a)	% (3b)	No. (4a)	% (4b)	No. (5a)	% (5b)	Mean (6a)	% (6b)	No. (7a)	% (7b)	No. (8a)	% (8b)	Mean (9a)	% (9b)
Untreated controls	14*	—	14*	—	13·8*	—	16	—	12	—	20·6	—	9	—	4	—	52·6	—
Castrated controls	42	+200	62	+343	3·7	−73	51	+215	69	+475	3·1	−85	16	+78	8	+100	31·5	−40
Testosterone	16	+14	11	−21	15·0†	+9	20	+25	17	+42	15·5	−25	9	0	4	0	53·4	+2
Methandrostenolone	16	+14	11	−21	14·8	+7	20	+25	16	+33	16·3	−21	8	−11	4	0	50·4	−4

No., Absolute number per unit area (5 cm × 5 cm).
%, Percentage change of untreated controls.
Mean, mean area of fibre cross-sections expressedi n arbitrary units.
* Each value represents the mean of 36 to 104 individual measurements.
† This value is significantly ($P < 0.01$) higher than the corresponding value of untreated controls.

cross-sections usually have an irregular polygonal configuration, measurement of their diameter is not a suitable criterion for estimating changes in their volume. The dependence of the 3-volume parameters already mentioned on the hormonal treatment is shown in Table 1. As was to be expected[6], castration led to a reduction in the volume of the muscle fibres, which was reflected in an increase in the number of cell nuclei and of muscle fibres per unit of area and in a decrease in the areas of the fibre cross-sections. The extent of this reduction in volume was most marked in the case of the m. bulbocavernosus, less marked in that of the m. levator ani, and least marked of all in that of the m. psoas.

The administration of testosterone and of methandrostenolone to castrated animals in the dosages indicated produced an increase in fibre volume in all the types of muscle studied (cf. Fig. 1); the reaction of these muscles to the hormonal stimulus, however, was not uniform. Thus, testosterone and methandrostenolone increased the fibre volume in the m. levator ani and in the m. psoas almost to the level of that in the untreated controls, whereas the values for the m. bulbocavernosus were lower. Both testosterone and, to a somewhat lesser extent, methandrostenolone caused the fibre volume of the m. levator ani, as estimated by measuring the area of the fibre cross-sections, to rise above the control-levels by 9 and 7 per cent respectively (Table 1).

The results of the interferometric estimation of the optical path difference are indicated in Table 2. It can be



Dry weight W	W_1	≫	W_2	≪	$W_3 (\simeq W_1)$
Area A (longitudinal section)	A_1	≫	A_2	≪	$A_3 (\simeq A_1)$
Volume V (a·l·t)	V_1	≫	V_2	≪	$V_3 (\simeq V_1)$ (Table 1)
Concentration $C = \frac{M}{V}$	C_1	≃	C_2	≃	C_3
Optical path difference φ	φ_1	=	φ_2	≃	φ_3 (Table 2)
$\frac{\text{Dry mass}}{\text{Area}} = \frac{M}{A} = \varphi$	$\frac{M_1}{A_1}$	≃	$\frac{M_2}{A_2}$	≃	$\frac{M_3}{A_3}$
Dry mass $M = \varphi \cdot A$	M_1	≫	M_2	≪	M_3

Fig. 1. Diagram showing the experimental procedure

Table 2. Dependence of the Optical Path Difference (f) on Hormonal Treatment

(Mean values ± $S.E.$)

Treatment	M. levator ani	M. bulbocavernosus	M. psoas (white)
Untreated controls	0·37 ± 0·032	0·36 ± 0·054	0·35 ± 0·039
Castrated controls	0·49 ± 0·088*	0·39 ± 0·143	0·45 ± 0·06*
Testosterone	0·39 ± 0·084	0·40 ± 0·141	0·41 ± 0·140
Methandrostenolone	0·33 ± 0·156	0·39 ± 0·079	0·36 ± 0·075

* These values differ significantly ($P < 0.01$) from those of the corresponding untreated controls.

seen that castration caused a slight increase in the optical path difference and thus in the dry weight/area of the muscle fibres (cf. Fig. 1). The administration of testosterone or methandrostenolone brings the optical path difference back again to within the vicinity of the control-levels.

The castration-induced increase in the optical path difference of muscle fibres appeared to be relatively small (Fig. 1). Since the area ($A2$) of the fibres is markedly reduced following castration, the optical path difference $\varphi \left(= \frac{M2}{A2}\right)$ would have to show a corresponding increase in relation to the untreated controls if the dry mass remains constant ($M1 = M2$). As, however, this is not the case (Table 2), it can be assumed that the muscle fibres must undergo an appreciable loss of substance as a result of castration and that the two hormones are capable of making good this loss (Fig. 1 and Table 2).

This loss of substance, demonstrated with the aid of interferometry, must also exert an effect on the concentration of dry substances in the muscle fibres. Since the considerable diminution in the volumes (Table 1), which occurs following castration, is accompanied by a loss of dry mass, the concentration in the muscle fibres of castrated animals does not undergo any substantial change in comparison with the untreated controls and the hormone-treated animals (Fig. 1). In the light of the proportional relationship between the optical path difference and the concentration[5] in our experimental procedure, we attempted to correct the path difference with the aid of the volume parameters listed in Table 1 (Table 3). It can be seen that the following expressions yield values which—

Table 3. Relationship between the Corrected Optical Path Difference and Dry Weight (as determined gravimetrically)

Muscle	Treatment	As percentage of untreated controls: φ*/(1a)	φ/(2a)	φx(3a)	Dry weight
M. levator ani	Castrated controls	44	31	35	38
	Testosterone	92	130	115	98
	Methandrostenolone	80	115	96	96
M. bulbocavernosus	Castrated controls	32	17	16	16
	Testosterone	86	79	84	86
	Methandrostenolone	86	83	86	88
M. psoas (white)	Castrated controls	68	69	77	—
	Testosterone	112	125	119	—
	Methandrostenolone	115	123	99	—

*φ (compare with Table 2) was corrected with the aid of the volume parameters listed in Table 1.

with a few exceptions to be dealt with later—tally reasonably well with the gravimetric estimation of the dry weight (Table 3): (1) ratio between optical path difference (φ) and number of cell nuclei per unit of area (1*a*, 4*a*, 7*a*, Table 1); (2) ratio between optical path difference (φ) and number of fibres per unit of area (2*a*, 5*a*, 8*a*, Table 1); (3) product of optical path difference (φ) and area of the fibre cross-sections (3*a*, 6*a*, 9*a*, Table 1). Among the various volume parameters, the optical path difference (expressed in terms of concentration of dry substance) appeared to be most directly related to the area of the fibre cross-sections.

Table 3 also shows that following testosterone treatment the corrected optical path difference (column 3*b*) of the m. levator ani and of the m. psoas is 15 and 19 per cent, respectively, above the corresponding values in the untreated controls; methandrostenolone does not display this effect.

The reason for this difference is not known. There are, however, two main possibilities:

(1) It is conceivable[7] that the hormone-stimulated synthesis of cellular substances, that is, contractile proteins, for example, proceeds more rapidly in the region of the cell nuclei at the periphery than in those zones of the fibres further removed from the nuclei. Hence, there would have to be a concentration gradient decreasing from the periphery towards the centre of the fibre. The optical path difference, however, was determined at the periphery of the fibre. If this gradient were to be maintained for longer following testosterone than following methandrostenolone, determination of the optical path difference should yield higher values for testosterone. The figures listed in Table 2 do in fact indicate that testosterone tends to raise the optical path difference in the 'white' muscles above the values found for methandrostenolone. Pointing in the same direction is the observation that following testosterone an inhomogeneous distribution of the myofibrils is encountered in stained histological cross-sections of the m. levator ani and m. psoas; this cytological picture could be due to the fact that hormonal stimulation of the sarcoplasm and its particulate organelles[4] has not yet been completely arrested[2] at this point (14 days after castration).

(2) The second possible explanation would be that determination of the optical path difference yields higher values following testosterone than following methandrostenolone (Table 2) because the dry mass of connective tissue (*D*, Fig. 1) is affected in different ways by the hormones which nevertheless otherwise exert the same type of effect on the muscle cell.

The results of interferometric measurements of individual muscle fibres are very closely related to those of gravimetric determinations of muscle mass. Although the interferometric method is rather laborious, it makes it possible to quantitate certain cytological changes which may be encountered as a result of hormone treatment.

We thank Prof. F. Ruch of the Federal Institute of Technology, Zurich, for his advice and Dr. P. Barthe for his help in preparing the animals for the experiments.

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A PHAGOCYTIC FUNCTION OF HASSALL'S CORPUSCLES

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In *The Microscopic Anatomy of the Human Body in Health and Disease*, published in 1849, Hassall[1] first described and illustrated the corpuscles which bear his name. He quotes from a 'Prize Essay' entitled *A Physiological Essay on the Thymus Gland* by John Simon, F.R.S. (Renshaw, London, 1845), who examined thymus glands from animals of varying maturity and concluded from "the application of acetic acid to the histological section", that the cells of the corpuscles had a "great affinity to embryonic cells". The origin and function of these corpuscles remain unknown or under dispute, and as recently as 1964 Christianna Smith[2], an authority on thymic anatomy, wrote: "The weight of evidence as to the origin of typical Hassall's corpuscles favours the theory stated by Kingsbury in 1928, that they are the expression of growth within a confined space of an epithelium which has lost its surface function not dissimilar to the epithelial pearls of keratinizing epitheliomas."

During an investigation of the entry of proteins, colloidal and particulate materials into the thymus of guinea-pigs under normal and pathological conditions, it was seen that Hassall's corpuscles were larger when the gland had involuted following either local X-irradiation or systemic cortisone. Two days after injecting indian ink into the circulation, particles of carbon were visible in macrophages inside the thymus of control animals and in greater concentration in the treated group. In addition, carbon was evident in some Hassall's corpuscles, and it is this phenomenon that is the special purpose of this article.

Young immature albino guinea-pigs (Hartley strain) of both sexes, weighing 220–370 g and 7–10 weeks old, were grouped into animals of comparable weight and sex. Each experimental animal had its own control. The thymus was caused to involute either by local X-irradiation or by systemic injection of cortisone.

Each animal was anaesthetized with intraperitoneal Standard Veterinary Pentobarbitone Sodium B.P. (Abbotts), laid on its back and the limbs secured. A lead screen covered the animal apart from a window, 4 cm × 5 cm, through which 300 r. of X-rays were directed to the ventral aspect of the neck. The irradiation constants were 40 r./min, 220 kV and half-value layer 1·4 mm copper. Five animals were irradiated, three of which were allowed to survive for 2 days and two for 7 days when they were killed. Five control animals were anaesthetized, not irradiated and killed at similar time-intervals. In the cortisone group, five animals received subcutaneous injections of cortisone acetate B.P. (Organon) twice daily; two had 2·5 mg for 5 days and three others had 5·0 mg for 7 days after which time they were killed. Five control animals had the same number of subcutaneous injections of sterile saline in the same volume, 0·1 and 0·2 ml.

Each animal, irradiated, cortisone treated or control, had one intracardiac injection of 0·1–0·15 ml./100 g bodyweight of indian ink (Günther Wagner Pelikan Werke, Hanover, Batch *C*11/1431*a*), which consisted of 10 per cent carbon black with an average particle size of 200–500 Å, stabilized with 4·3 per cent fish glue, and contained 1 per cent phenol in water. The injections were given within 1 h of irradiation or at the commencement of the series of cortisone injections, with the exception of one

irradiated animal and one control animal which were injected 2 days after the initial time and killed 1 h later.

The guinea-pig's thymus, which is situated in the neck, cannot be injured by intracardiac puncture.

The animals were killed with ether anaesthesia and the tissues immediately fixed in 10 per cent formol saline. Sections, 5–7μ thick, were cut from each lobe of the thymus, a cervical and mesenteric lymph node, spleen and from either the lung or the liver. Sections stained with haematoxylin and eosin did not provide good contrast for the small carbon particles. For this purpose light staining with methylene blue or neutral red was better.

All the animals were seen to change to a slaty grey colour during the injection of indian ink, and to return to their normal colour within minutes. At post-mortem the spleen from each animal was intensely black and the liver darker than normal. Some lymph nodes were darkened in patches. The thymus glands from animals that had been exposed to X-rays or treated with cortisone were darker than those from control animals which were pink. The treated group showed varying degrees of diminution in size of the thymus, and both groups had occasional black streaks on their surfaces.

Histologically, the spleen from each animal contained very large quantities of carbon in the sinuses of the organ. Large amounts of carbon particles were also to be seen in all the livers examined (irradiated group) and a considerable amount in the lungs (cortisone group). The lymph nodes, one cervical and one mesenteric from each animal, contained carbon particles albeit to varying extents.

After irradiation, thymic lobules showed the 'inverted' relationship of cortex to medulla, the cortex staining less deeply owing to impoverishment of thymocytes and prominence of reticulum cells. Following cortisone treatment the cortex was narrower than in control animals and showed the 'pockmarked' appearance. These cavities contained macrophages. The thymus glands from all treated animals contained a large number of Hassall's corpuscles, their size at times reaching very large dimensions.

Particles of carbon were evident in blood-vessel walls inside the gland as well as between and on the surface of lobules. The uniformly small particles in the wall of blood vessels contrasted with their size in macrophages where they ranged from small to large and in many instances had aggregated into sizable masses (Figs. 1 and 3). These heavily laden macrophages were to be found in the capsule, cortex, cortico-medullary junction and the medulla. In the cortex they frequently occupied the cavities of the 'pockmarking'. At the cortico-medullary junction where the blood vessels were dilated they were situated perivascularly. In the medulla some were close to, and others well away from, blood vessels. The concentration of carbon-bearing macrophages at the junction of the cortex and medulla was striking in some cortisone-treated animals and contrasted with the occurrence, often in patches, of solitary labelled macrophages both in the cortex and medulla in the irradiated group. In all the treated animals the quantity of carbon was much greater than in control groups, and comparison of the whole series showed that, the longer the time that had elapsed between injection of indian ink and death, the more carbon was present in the gland.

The Hassall's corpuscles consisted of a hyaline lamellated portion resembling a cut onion. Associated with this was a variable amount of deeply staining chromatin material consisting of small nuclear fragments, their differing shapes resembling polymorphonuclear leucocytes. In control animals this 'compound' corpuscle occurred far less frequently and was invariably smaller than in involuted glands. There they gave the appearance of having resulted from the fusion of two and often more corpuscles and were very large (Fig. 3).

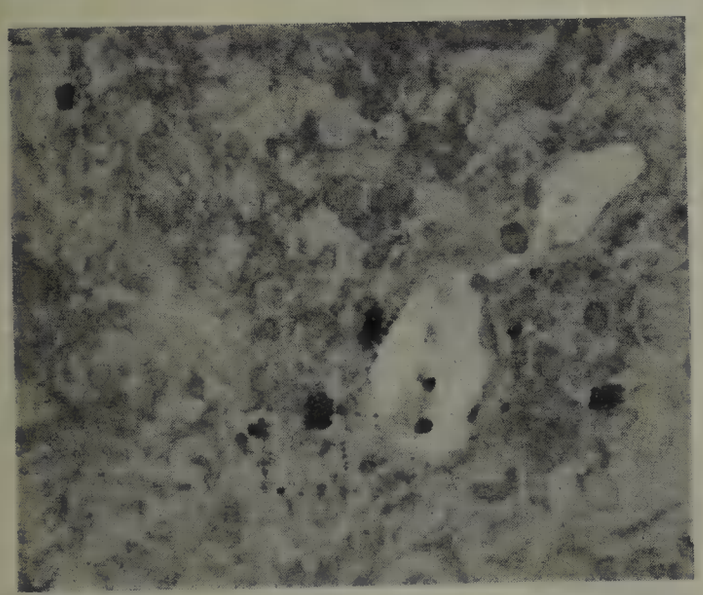

Fig. 1. Blood vessel at cortico-medullary junction from an animal treated with 2·5 mg cortisone twice daily for 5 days showing carbon in macrophages inside dilated vessel. Fine particles of carbon are seen in vessel wall and larger aggregates in macrophages adjacent to and away from the vessel (methylene blue, × 540)

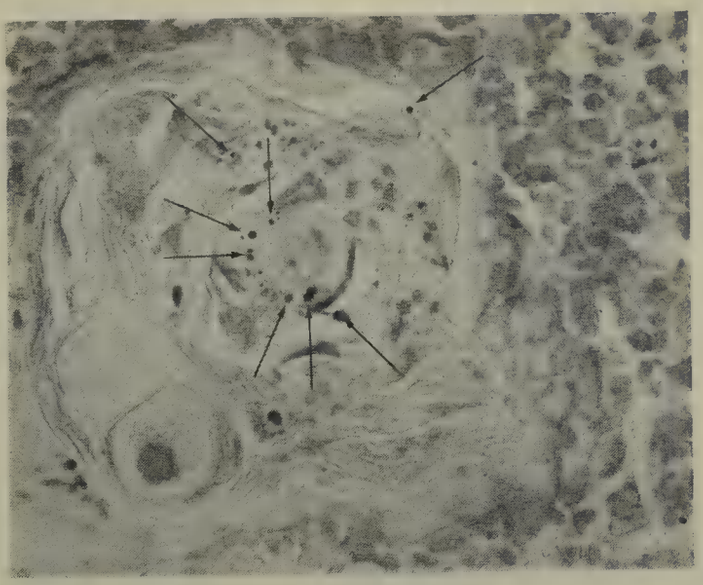

Fig. 2. Hassall's corpuscle containing carbon particles of varying sizes. Animal irradiated with 300 r. locally over the thymus and indian ink injected intravascularly 7 days previously. Carbon particles, indicated by arrows, have to be distinguished from deeply staining nuclear debris (methylene blue, × 540)

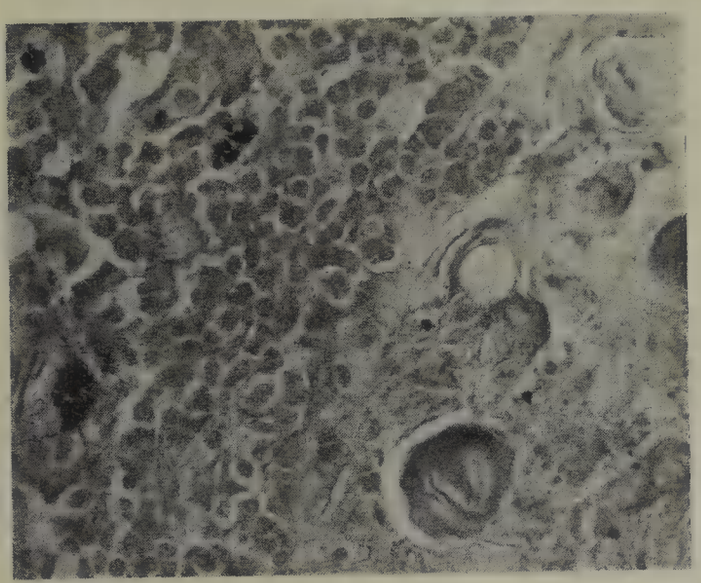

Fig. 3. From same animals as Fig. 2, showing the appearance of a 'compound' Hassall's corpuscle containing carbon. A heavily laden perivascular macrophage and 3 other macrophages not proximate to a vessel are visible (methylene blue, × 540)

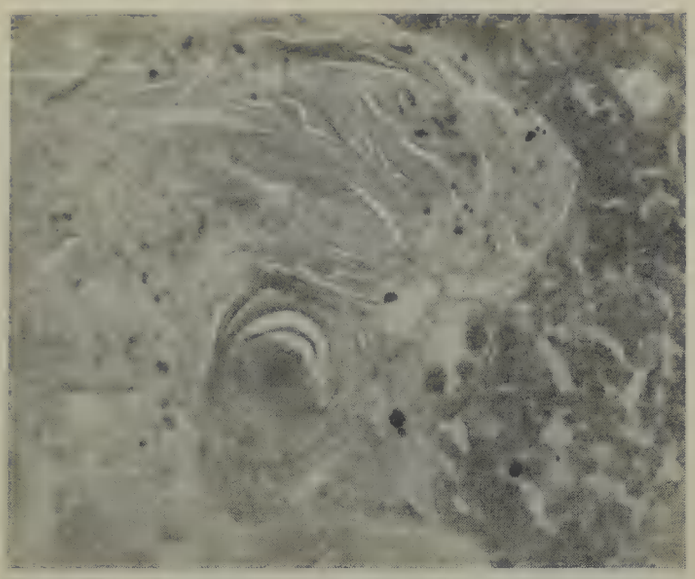

Fig. 4. Hassall's corpuscle from an animal injected with 5 mg cortisone twice daily for 7 days. Carbon is seen outside, at the edge and inside the corpuscle (methylene blue, × 540)

Carbon was rare in the lamellated portion of the corpuscle, but was evident in varying quantities and size of particle among the nuclear debris (Fig. 4). By no means did all the corpuscles in stimulated animals contain carbon, yet some control animals had a few of these 'compound' corpuscles which contained a little carbon, suggesting that involution was going on. At the periphery of a corpuscle a large cell with foamy cytoplasm was seen at times to contain one or more particles of carbon. Here as well as inside the corpuscle the variable size of the particulate matter corresponded to the variation found in macrophages (Fig. 4).

The striking morphological resemblance of Hassall's corpuscles to keratinized squamous epithelium has perhaps been one of the mainstays of the belief that they are vestigial remnants arising from epidermal invagination from the third and fourth branchial pouches. The human embryos on which Norris[3] based his conclusions were examined by Ruth *et al.*[4] in the Embryology Department of the Carnegie Institute, Washington. The latter stated that: "Norris is the only investigator who claims to have traced the development of these structures to ectoderm. His drawings are interesting but not convincing."

So far the views held on the origin of Hassall's corpuscles have obscured investigation of their potential active function. Thus, well-recognized observations of their increase in size following irradiation[5] and systemic cortisone[6] have been neglected. The increase in content of nuclear fragments noted in the corpuscles during involution of the thymus gland cannot be accounted for on the basis of an effete vestigial structure, neither can the presence of γ-globulin in the corpuscles of human infants, children[7], normal adults and patients with myasthenia gravis[8].

If Hassall's corpuscles are not vestigial structures, what then is their function? Kostowiecki[9] argues that macrophages exist in the thymus and lists an impressive series of authors supporting his views. Green and Bloch[10] showed that carbon particles appeared in macrophages in some new-born mice, Clark[11] confirmed the entry of ferritin in adult mice and Bailliff[12] found the thorotrast in the thymus of rats and mice. The experiments cited here, using carbon, confirm this in the guinea-pig, and other investigations with Evan's blue (unpublished) are also in agreement.

The suggestion that macrophages become incorporated and form Hassall's corpuscles has been made by Kostowiecki[9], who found that following the intraperitoneal injection of Evan's blue the corpuscles were stained with dye. Clark[11], too, found this dye to be present in some cells which formed rudimentary corpuscles in mice.

The observations reported here demonstrated that particles of carbon are present in macrophages and in Hassall's corpuscles if sufficient time is allowed to elapse after intravascular injection, and this effect was markedly accentuated if the gland was caused to involute. This function of the corpuscle has hitherto been hinted at, but not satisfactorily demonstrated for a number of possible reasons: use of the wrong animals, like rats and mice, where the corpuscles are rudimentary; use of normal animals in which the thymus gland is not involuting or involution is proceeding very slowly; failure to use a readily visible foreign particle; allowing insufficient time for the accumulation of foreign material in the gland. It is only the simultaneous presence of these four conditions which has made these observations possible. This may explain why in Marshall and White's experiments[13] trypan blue and pneumococcal polysaccharide failed to enter the normal gland, yet gained entry when the gland was traumatized: hence their proposition of a blood-thymus barrier. Clark[11] supports the concept of a functional and partial barrier and the observations cited here would be in favour of such a barrier rather than an absolute one, since particulate matter, ferritin in mice and carbon in guinea-pigs, has been found in macrophages of unstimulated animals.

The mode of entry is almost certainly via the blood stream. In comparing sections from animals that had carbon injected 1 h, 2, 5 and 7 days before death, there were increasing quantities of particulate matter in macrophages situated perivascularly and well away from blood vessels. Hence a likely interpretation is that perivascular macrophages take up carbon from the walls of the blood vessels which they line, or possibly circulating macrophages migrate through these vessels, as is suggested by Fig. 1. The permeability of the small blood vessels of the thymus may well have been affected by irradiation and cortisone treatment, and certainly in the latter group vessels seemed more prominent. These perivascular macrophages might then migrate to the Hassall's corpuscles and contribute in their formation.

Whether this dynamic interpretation derived from a series of static histological sections, taken at timed intervals, is correct or not requires further evidence. However, if the definition of a reticulo-endothelial tissue is based on its ability to phagocytose foreign particulate matter, then the demonstration of carbon black inside Hassall's corpuscles must imply that they belong to this tissue system in the guinea-pig.

To what extent are these observations relevant to the behaviour of the thymus in man in health and disease? The illustrations of Hassall's corpuscles in children with involution of the thymus[14] bear such close similarity to those seen in the guinea-pig after irradiation and systemic cortisone that one can see little, if any, difference. The presence of germinal centres in some patients with myasthenia gravis and in a strain of New Zealand mice[15] implies that an antigenic stimulus has reached the organ. Furthermore, Marshall and White[13] produced germinal centres in the guinea-pig thymus by direct injection of killed typhoid and paratyphoid bacilli into the exposed thymus gland. Hence it is possible that, if antigenic molecules reach the thymus via macrophages in sufficient concentration, this could provide the stimulus for the formation of germinal centres. The local conditions in the thymus which determine the formation of these centres awaits elucidation.

In conclusion, Hassall's corpuscles in the thymus glands of guinea-pigs have been shown to take up particles of carbon after the injection of indian ink into the circulation, especially when the thymus glands are undergoing involution caused either by X-irradiation or the systemic injection of cortisone. It is suggested that macrophages play an important part in the formation of Hassall's corpuscles which in certain circumstances have a phagocytic function and form a part of the reticulo-endothelial system.

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ANDROGEN–OESTROGEN-INDUCED TUMOURS: LACTIC DEHYDROGENASE RELATIVE TO CHAETEPITHELIOMA INDUCTION IN THE SYRIAN HAMSTER

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THERE is some presumptive evidence that neoplastic changes (induction) and subsequent progression from the benign to the malignant state may be accompanied by fluctuations in enzyme patterns. In view, especially, of the accumulating data suggesting a possible relationship between lactic dehydrogenase (LDH) and progression in certain tumours[1], this enzyme has been selected for an initial study on the histogenesis and growth of an androgen–oestrogen-induced, hormone-dependent epithelioma (chaetepithelioma) deriving from matrix cells of hair follicles in the flank organs (scent glands) of the Syrian hamster[2].

The chaetepithelioma is a particularly favourable tumour for use in such an enzyme investigation because of the essential role of sex hormones in the clear-cut succession of developmental stages[3]. Under endogenous androgen influence the tumour arises from epithelial cells migrating from hair bulbs into adjacent connective tissue. These cells organize the connective tissue into microscopic, laminated, epithelial-connective tissue complexes (stage 1) which, under the influence of exogenous androgen stimulation, enlarge to barely macroscopic, still preneoplastic stage 2 nodules. Stage 2 masses enlarge and fuse to form potentially malignant stage 3 neoplasms only following the addition of oestrogen to the androgen[2].

Initially this survey of LDH has been limited to tumour-bearing animals treated with both the sex hormones required for progression to ultimate malignancy. Tissue samples were taken at appropriate intervals during the life-history of the neoplasm for isoenzyme histochemistry using microscopic methods as well as acrylamide-gel electrophoresis.

Each histological tissue sample was sectioned serially at 20μ on an International Harris cryostat. Both fresh and acetone-fixed sections from each sample were incubated for 10–20 min in 0·1 M DL-lactate LDH medium. Since available sulphydryl (S–H) groups are known to reduce nitro-blue tetrazolium, fresh sections were incubated in the standard LDH medium containing the S–H blocking agent, *N*-ethyl-maleimide. Each of these procedures was controlled by omitting the substrate from the incubation medium. Definitive tumour tissue (stage 3) was subjected to electrophoresis on acrylamide-gel strips according to Brody[4]. Urea (2·6 M concentration) was used to depress the slowest migrating isoenzyme[5] in tissue sections.

LDH assessments were made on three groups of androgen–oestrogen-treated animals selected after periods of treatment known to evoke the different stages of tumorigenesis[2], that is, 100–175 days for stage 1, 150–250 days for stage 2, 250 days or longer for stage 3. Histologically, the flank organ hair matrices of the short-term androgen–oestrogen-treated animals contain variable amounts of LDH ranging from slight to relatively heavy concentrations. Smaller, though less-variable, amounts of LDH occur in the outer root sheaths of the hair bulbs. Connective tissue components (dermal sheaths and papillae) always contain recognizable amounts. Laminated corpuscles (stage 1) contain recognizable quantities of LDH concentrated predominantly in the central core composed of epithelial cells. Little LDH is seen in the connective tissue laminae although scattered cells may contain as high a concentration as is present in any single cell in the central aggregate.

Stage 2 tumours, in which connective tissue components are reduced to a supporting stroma for cords of epithelial cells[2], contain greater concentrations of LDH than do stage 1 nodules, although considerable variation persists in different samples. With the transformation of stage 2 to stage 3 masses, the variability in amounts of LDH in different samples largely disappears and the concentration becomes distinctly greater.

Acrylamide-gel electrophoresis of stage 3 tumour tissue discloses four distinct bands of LDH. Of these, the most conspicuous is the slowest-moving isoenzyme, LDH No. 5 (ref. 4), with decreasing amounts of No. 4, No. 3 and No. 2. If the fastest-moving isoenzyme (No. 1) is present, its concentration is below the sensitivity threshold of this method. Attempts to isolate sufficient quantities of tissue from stages 1 and 2 for electrophoretic determinations have not yet been successful. These results are summarized in Figs. 1 and 2.

A 2·6 concentration of urea, which suppresses the slow-moving band (No. 5) (ref. 4) on acrylamide gel, is found in histological preparations to depress the LDH complement in the flank organ hair matrices, to completely inhibit the enzyme in stage 1 tumours, to depress the amount in stage 2 tumours to varying degrees and to diminish uniformly the reaction in stage 3 tumours. The order of magnitude of this latter inhibition is approximately 50 per cent which is roughly comparable to the percentage inhibition seen in the hair matrices.

It appears from this preliminary investigation that LDH diminishes during the initiation of stage 1 nodules, and that there is a gradual and variable synthesis of the

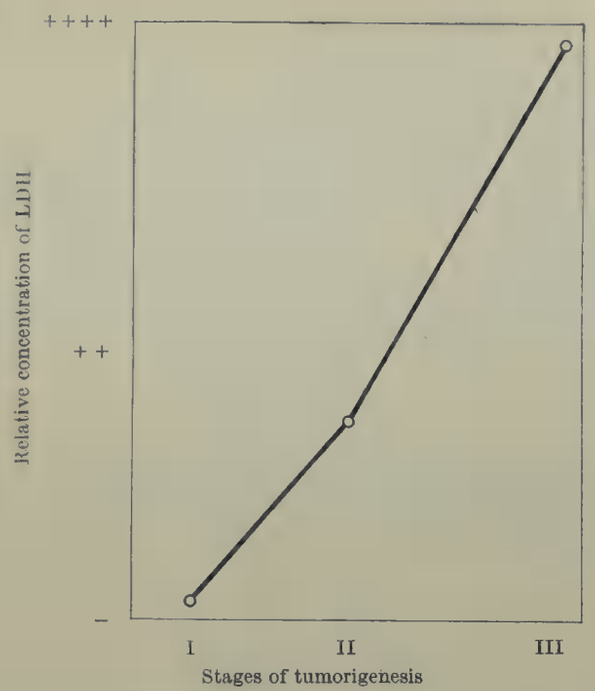

Fig. 1. Comparison of relative amount of LDH per section of the three tumour stages

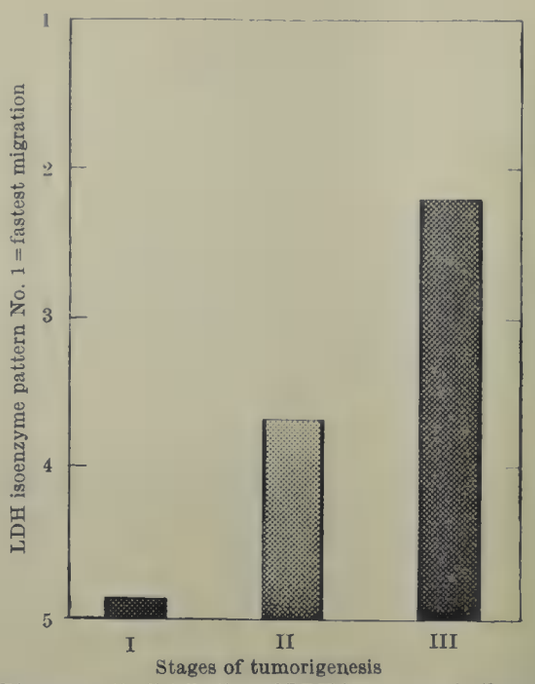

Fig. 2. Diagrammatic distribution of LDH isoenzymes in three tumour stages based on 2·6 urea inhibition investigations of sections and acrylamide-gel electrophoresis

enzyme, probably in more than one form, during the transformation of stage 1 to stage 2 masses and ultimately the marked elaboration of additional types and quantities of LDH during the development and growth of the definitive stage 3 tumour.

The full physiological significance of this investigation necessarily awaits the completion of similar studies of untreated males and females of varying ages, as well as animals treated independently with each of the two hormones. Of value, also, would be an investigation of the effect on types and levels of LDH in these tumours following hormone withdrawal. The correlation of hormone treatment with LDH synthesis during the life-history of this epithelioma may be physiologically significant, *in vivo*: whether there may be a direct influence on types and concentrations of LDH isoenzymes might, however, be best determined using *in vitro* techniques[6–8]. The possible involvement of viral LDH stimulating agents[9–13] must await additional studies.

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TITRATION CURVES DEPICTING CENTRAL STIMULANT, DEPRESSANT AND DUAL STIMULANT-DEPRESSANT ACTION OF GLUTARIMIDE HOMOLOGUES IN MICE

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EVIDENCE has been presented from quantitative titrations in mice that certain analeptics (including bemegride) and hypnotics (including pentobarbitone sodium) act at common sites in the central nervous system[1,2]. It has been proposed[1], in terms of 'rate theory' of drug action[3], that with respect to the complex formed with the responsive sites, an analeptic is an agonist with high association and dissociation rate constants (k_1 and k_2, respectively) and thereby maintains a continued high rate of association with the sites which manifests as convulsion. On the other hand, a hypnotic has a low k_2 and prevents the action of the agonist by its prolonged association with the responsive sites. Analepsis occurs when the agonist maintains a sufficiently high rate of association in the presence of hypnotic to overcome the depression caused by the latter. The action of these drugs has also been described[1] in terms of 'receptor occupation theory' of drug action[4].

Independent support for these views has been obtained from structure/action investigations in mice with members of a homologous series of β,β-disubstituted glutarimides. It has been shown that β-methyl-β-ethylglutarimide (bemegride, 'Megimide'), β-methyl-β-*n*-propylglutarimide and β-methyl-β-*n*-butylglutarimide and β-methyl-β-*n*-amylglutarimide show the properties of agonist, partial agonist and antagonists respectively in the central nervous system; the partial agonist, which is intermediate in structure between agonist and antagonist, has dual stimulant and depressant action and, it is proposed, an intermediate k_2. It is probable that these substances,

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Table 1. Median Convulsant and Hypnotic Doses and Least Squares Regression Lines representing Titration* by Pentobarbitone Sodium of Homologous β,β,Diakylglutarimides in Mice

β,β-Dialkyl glutarimide	Regression lines representing stimulant action	Regression lines representing depressant action	Median convulsant dose (μM/kg)	Median hypnotic dose (μM/kg)
Methyl, ethyl (bemegride)	$y = 0{\cdot}2149\,x + 110{\cdot}7$ (lower doses) $y = 0{\cdot}0556\,x + 177{\cdot}7$ (higher doses)	—	126·8 (123·0–137·6)†	—
Methyl, *n*-propyl	$y = 0{\cdot}1027\,x + 112{\cdot}9$ (lower doses)	$y = -0{\cdot}0898\,x + 214{\cdot}1$ (higher doses)	227·8 (227·1–228·5)†	2,232 (2,181–2,259)†
Methyl, *n*-butyl	—	$y = -0{\cdot}0835\,x + 108{\cdot}0$	—	1,340 (1,316–1,354)†
Methyl, *n*-amyl	—	$y = -0{\cdot}1578\,x + 106{\cdot}7$	—	676 (667–683)†

* The titration end-point is hypnosis in 50 per cent of a group of mice.

† Estimated limits, $P = 0{\cdot}05$.

which differ only by the progressive addition of one methylene group to the β-alkyl substituent, and the hypnotic pentobarbitone sodium all act at common central sites which mediate both the stimulant and depressant actions of these drugs[5].

If these views are correct, it may be anticipated that when progressively increasing doses of each of the aforementioned glutarimides are titrated against pentobarbitone sodium to an end-point of hypnosis in 50 per cent of a group of mice, the agonist will show only increasing analeptic activity, the partial agonist initial analeptic activity followed by potentiation of hypnosis, and the antagonist only increasing potentiation of hypnosis. Titration curves substantiating these views are shown in Fig. 1. In carrying out these titrations the titrants, either in aqueous solution (pentobarbitone sodium, bemegride and the *n*-propylglutarimide) or as a 1 per cent tragacanth suspension (the *n*-butyl- and *n*-amyl-glutarimides), were administered intraperitoneally in a temporal sequence such that their maximal effects occurred contemporaneously. The titration interval following drug administration was 10–15 min. Each titration point is the mean of at least two concordant estimations.

Theoretically, it is expected that the titration points for bemegride and its *n*-propyl homologue against pentobarbitone sodium take the form of hyperbolae[6], but their relative analeptic potency has been estimated by fitting the points in each case by two highly significant least squares regression lines ($P < 0{\cdot}001$) which intersect and show highly significant difference in slope ($P < 0{\cdot}001$); each lower regression line intersects the hypnotic axis at a point non-significantly different from (0, HD_{50}) (Fig. 1 and Table 1).

It may be seen (Table 1) that bemegride is approximately twice as potent as its *n*-propyl homologue both as an analeptic to pentobarbitone sodium depression and as a convulsant. Their relative analeptic potency was calculated from the slopes of the lower regression lines, that is, where increasing doses of either glutarimide show

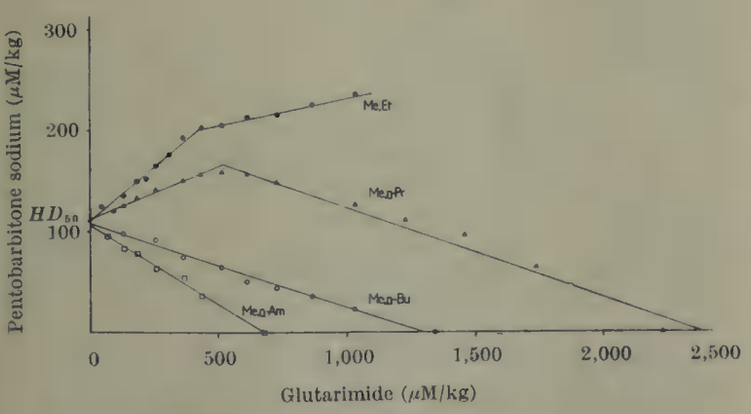

Fig. 1. Titration curves and least squares regression lines representing titration of pentobarbitone sodium by β-methyl-β-ethylglutarimide (Me,Et), β-methyl-β-*n*-propylglutarimide (Me,*n*-Pr), β-methyl-β-*n*-butylglutarimide (Me,*n*-Bu) and β-methyl-β-*n*-amylglutarimide (Me,*n*-Am). The titration end-point is hypnosis in 50 per cent of a group of mice. Each titration point is the mean of at least two concordant estimations

increasing antagonism to barbiturate depression, while their relative convulsive potency was calculated from the CD_{50} values (Table 1), estimated by plotting log. dosage of drug against probit percentage of mice which show mild clonic convulsion. By analogy with Paton's *in vitro* findings with homologous alkyltrimethylammonium cations[3], it is likely that the k_1 values of bemegride and its *n*-propyl homologue are very similar and, hence, that the latter has a higher affinity (k_1/k_2) for the common responsive sites since, being a partial agonist, its k_2 is lower. It is also likely, judging by the close similarity in their structures and time courses of action, that a similar proportion of the peripheral dosages of the two glutarimides will reach the responsive sites during the titration interval. Consequently, it appears that k_2 of the drug–site complex is the cardinal factor determining the potency of these two drugs as stimulants in the central nervous system; this is to be expected from the assumption that k_2 limits the rate of dissociation of the drug from the responsive sites and hence its rate of association with the sites, and from Paton's definition of stimulant potency (k_2/k_1)[3].

The inflexion in the bemegride–pentobarbitone sodium titration curve (Fig. 1) is considered to represent approaching saturation of the common sites for which the hypnotic has the greater affinity[1,2]; a greater concentration of analeptic is required to attain the titration end-point in the presence of a hypnotic with a higher affinity when the drugs are present in excess with respect to the responsive sites than when the number of sites is adequate for maximal association of both drugs at the concentrations present. It is likely that the inflexion in the β-methyl-β-*n*-propylglutarimide–pentobarbitone sodium titration curve, in this case a change from a positive to a negative slope, also represents approaching saturation of the responsive sites since, in these circumstances, the stimulant action of the partial agonist would be expected to decline because its k_2 would be too low to permit the maintenance of an adequate rate of association when the number of free sites is limited.

The titration points of the *n*-butyl- and *n*-amyl-glutarimides against pentobarbitone sodium have also been fitted by least squares lines which show highly significant regression ($P < 0{\cdot}001$) and which approximate very closely to the lines joining the estimated HD_{50} values of pentobarbitone sodium, 110·4 μM/kg (109·2–111·0 μM/kg, $P = 0{\cdot}05$), and β-methyl-β-*n*-butylglutarimide or β-methyl-β-*n*-amylglutarimide (Fig. 1 and Table 1) These HD_{50} values were estimated by plotting log dosage of drug against probit percentage of mice which lost righting reflex for at least 3 min.

Comparison of the slopes of the regression lines for the glutarimide hypnotics with that of the regression line for the higher doses of the partial agonist β-methyl-β-*n*-propylglutarimide shows that the *n*-amylglutarimide has approximately twice the depressant potency of its *n*-butyl homologue which is approximately equipotent to the *n*-propyl homologue (Table 1). However, the regression coefficient for the *n*-propylglutarimide gives a false

impression of its depressant potency since the regression line represents both decreasing analepsis, up to a dosage of 1,227 μM/kg, and potentiation of pentobarbitone-induced hypnosis at dosages beyond 1,227 μM/kg. A more accurate indication of the depressant potency of β-methyl-β-*n*-propylglutarimide is given by its HD_{50}, which was estimated by the same method already described here for the other glutarimides using a warm, near-saturated, aqueous solution (12 mg/ml.). A comparison of the HD_{50} values of the three glutarimides (Table 1) shows that β-methyl-β-*n*-amylglutarimide has approximately twice the depressant potency of its *n*-butyl homologue which is in turn approximately twice as potent as the *n*-propylglutarimide. This approximate two-fold difference in depressant potency for these drugs is similar to that shown for the stimulant potency of the ethyl and *n*-propyl homologues (Table 1) and probably reflects, primarily, a progressive fall in the k_2 of the drug–site complex representing, with each additional methylene group, the formation of a stronger bond between the glutarimide and the responsive sites.

It might be argued that potentiation of pentobarbitone sodium hypnosis by the higher doses of β-methyl-β-*n*-propylglutarimide results not from the action of the latter as a partial agonist but from its capacity to replace the barbiturate at common sites on plasma or tissue proteins or to decrease the rate of excretion, biotransformation or the extent of storage of the barbiturate, each of which would tend to increase the concentration of free hypnotic in the plasma for transfer to the responsive central sites. However, these possibilities seem unlikely since the *n*-propylglutarimide would need to be markedly more specific in these actions than its closely related ethyl, *n*-butyl and *n*-amyl homologues. The ethyl homologue, bemegride, is bound negligibly to plasma protein[7] and shows continued analeptic activity against pentobarbitone sodium over a 117-fold range of analeptic dosage[2], while the hypnotic *n*-butyl and *n*-amyl homologues produce no more than the expected additive depression in combination with the barbiturate.

Further, it has been demonstrated that whereas mice given β-methyl-β-*n*-propylglutarimide 500–600 μM/kg, in the absence of hypnotic, may die within 2–15 min of convulsion usually associated with maximal hindleg extensor tonus, animals receiving five times this dosage lose righting reflex within 2 min, following an initial burst of convulsion, and sleep without signs of stimulation for a period varying from 4 to 30 min. These mice wake with generalized hypertonia and excitement and show sporadic episodes of mild jumping and clonic movements the intensity of which decreases progressively until complete recovery some 3–4 h later. These observations suggest that β-methyl-β-*n*-propylglutarimide is a partial agonist which in high dosage can itself inhibit the maximal lethal convulsive action which it produces in a much lower dosage. Further, even though the drug is probably present for a considerable period at a concentration in excess of that necessary for maximal stimulation, it appears that the relationship between the k_1 and k_2 of the drug–site complex is such that the number of free responsive sites is never sufficiently high to maintain a rate of association greater than that commensurate with mild clonic convulsion.

Not only does β-methyl-β-*n*-propylglutarimide antagonize its own convulsant action but it also antagonizes that of its ethyl homologue (bemegride) and pentylenetetrazol. The *n*-propylglutarimide at a sub-hypnotic (1,800 μM/kg) or hypnotic dosage (Table 1) eliminates maximal hind-leg extensor tonus[8] produced in mice by a 100 per cent effective intraperitoneal dosage of the ethyl homologue (290 μM/kg) or pentylenetetrazol (800 μM/kg); mice receiving 1,800 μM/kg of the *n*-propylglutarimide show only mild stimulation and at times appear almost normal. Anticonvulsive action and potentiation of barbiturate depression by other compounds with stimulant properties have been reported previously, for example, 5,5-diphenyl-2,4-diketooxazolidine and its hydantoin analogue ('Dilantin')[9] and phencyclidine and the related compound '*CI*-581'[10]. Antagonism by high doses of stimulant action present in low doses has also been reported for the last-mentioned two compounds[10].

As anticipated, β-methyl-β-*n*-propylglutarimide in low dosage (114 μM/kg, $0{\cdot}5 \times CD_{50}$) potentiates the convulsive action of the ethyl homologue, decreasing its CD_{50} from 126·8 to 63·2 μM/kg (62·7–63·7, $P = 0{\cdot}05$); both drugs in a non-convulsive dosage ($0{\cdot}5 \times CD_{50}$) are analeptic to the *n*-butyl and *n*-amyl homologues at their HD_{50}. On the other hand, a higher dosage of the *n*-propylglutarimide (1,800 μM/kg) potentiates the action of the HD_{50} of the hypnotic glutarimides.

The dual stimulant and depressant action of β-methyl-β-*n*-propylglutarimide and the form of its titration curve suggest that stimulation, manifesting as convulsion or analepsis, and depression, manifesting as anticonvulsive action and potentiation of hypnosis or hypnosis, are continuous phenomena. Thus, it is very likely that convulsion and hypnosis are the opposed extremes of a common physiological process occurring at the responsive central sites; the pure convulsive and hypnotic actions respectively of the ethyl and *n*-butyl homologues strongly support this contention. A hypothesis has been presented[1,2] which suggests that convulsion and hypnosis produced by selected barbiturates, glutarimides and a variety of related drugs are the opposed extremes of physiological wakefulness occurring predominantly at excitatory synapses on reticular neurones concerned in arousal, and that anticonvulsive action is an intermediate state of depression. These views are consistent with those obtained from direct neurophysiological and neuropharmacological investigations[11] and with the ability of most anticonvulsive drugs to antagonize convulsion in low dosage and to produce hypnosis in higher dosage.

The relative affinities, association and dissociation rate constants and the central nervous system penetrabilities of pentobarbitone sodium, bemegride and other hypnotic and analeptic drugs[1] are being estimated (using the IBM 7044 computer) from titration curves of the type shown for bemegride and pentobarbitone sodium in Fig. 1 (ref. 6). These parameters, representing the action of the drugs at the common sites in the central nervous system of mice, will also be computed for the *n*-propyl, *n*-butyl, *n*-amyl and related glutarimide homologues from appropriate titration curves. It is hoped in this way to determine the progressive changes in the magnitudes of the association and dissociation rate constants which accompany the progressive addition of one methylene group to the β-alkyl substituent of the glutarimide ring. Similarly, changes in the penetrabilities of successive homologues can be examined and compared with changes in relevant physical characteristics.

It is suggested that the foregoing conclusions, concerning the central actions of β-methyl-β-*n*-propylglutarimide, and titrations of the type shown in Fig. 1 may be useful in elucidating the mode of action of other central nervous system stimulant and depressant drugs, especially those having apparently paradoxical actions. For example, these considerations have been applied to the central actions of a convulsant barbiturate and the anticonvulsant diphenylhydantoin sodium ('Dilantin'), and it seems possible that these substances, phencyclidine and '*CI*-581'[10], a number of psychoactive drugs[12] and certain halogenated aliphatic ethers and paraffins[13] also behave as partial agonists in the central nervous system and act at the same central sites common to the other drugs discussed in this article.

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BREEDING A PELAGIC COPEPOD, *Euterpina acutifrons* (Dana), IN THE LABORATORY

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IN order to evaluate the uptake, distribution and turnover of radioisotopes in a marine ecosystem, it is highly desirable to evaluate results observed in the open sea in terms of experiments carried out under precisely controlled conditions in the laboratory. An essential prerequisite is therefore to have at hand a method for rearing relevant organisms in the laboratory.

Copepods form an essential element in the marine food chains. So far, however, laboratory work with pelagic copepods has always posed serious problems, for it has been impossible to rear them under culture conditions which permit them to reproduce.

Only recently, Zillioux and Wilson[1] succeeded in maintaining a culture of the coastal species *Acartia tonsa* through five generations, and Conover[2] had previously been able to raise eggs of *Calanus hyperboreus* to the adult stage; these, however, failed to reproduce.

The excessive growth of bacteria in the culture vessels is presumed to be one of the main factors for the difficulties; Conover[2] therefore added antibiotics to the water. In order to bind heavy metal ions present, Bernhard[3] used EDTA and other complexing agents and was thereby able to overcome the difficulties in culturing sea urchin larvae.

Taking advantage of this experience, we have carried out breeding experiments with some species of pelagic copepods, especially with *Temora stylifera*, *Oncaea* sp. and *Euterpina acutifrons*, which are abundant in the Mediterranean. The copepods came from the laboratory's sampling zone (see Bernhard *et al.*[4]) in the Ligurian Sea, about ten nautical miles off the coast near La Spezia; the samples were taken in surface tows over a water depth of about 250 m, except for the individuals of *Euterpina acutifrons*, used to set up the first laboratory culture, which originated from the Gulf of La Spezia in the immediate vicinity of the coast.

Aboard the ship, the plankton samples were diluted with natural sea-water and kept in 5-l. polyethylene bottles to which 50 mg/l. penicillin was added. The samples were then stored at 18° C. During the 20 min required to transport the plankton from the quay to the laboratory, no further precautions were taken to keep the temperature constant. Immediately on arrival, the copepods were transferred to culture vessels and kept in a room thermostatically controlled at 18° ±1° C.

Natural sea-water served as basis for the culture media in all cases. EDTA in a concentration of 37 mg/l. ($=10^{-4}$ mol) was added as a complexing agent. As antibiotics, penicillin and streptomycin were used alternately; two different concentrations were tested: 50 mg/l. and 6·5 mg/l. The control media contained EDTA but no antibiotics.

A suspension of a mixture of the flagellates *Tetraselmis micropapillata*, *Dicrateria* sp. and *Platymonas* sp., a dinoflagellate *Gymnodinium* sp. and the diatom *Phaeodactylum tricornutum* was added as food. The culture medium was changed at regular intervals, using a siphon fitted with nylon gauze to prevent the nauplii from escaping; the intervals ranged from 1 week to 1 month according to the experiments.

The copepods were kept either individually as 'single cultures' in Boveri capsules with a capacity of 30 ml. or in groups of more than ten individuals as 'mass cultures' in Erlenmeyer flasks ranging in capacity from 2 l. to 10 l. The flasks were filled to half capacity or less.

In preliminary survival experiments with single cultures of *Temora stylifera* and *Oncaea* sp., two concentrations of antibiotics were compared: 6·5 mg/l. penicillin, in weekly alternation with 6·5 mg/l. streptomycin, gave better results than either the controls or the higher concentration of 50 mg/l. which was recommended by Conover[2].

The superiority of the lower concentration of antibiotics was significant at the 95 per cent level for *Temora* ($F = 3{\cdot}73$ at *d.f.* 2 and 28). For *Oncaea*, although it also survived longer in the lower concentration, the difference was not statistically significant ($F = 1{\cdot}71$ at *d.f.* 2 and 34). A mixture of various zooplankton species in a mass culture lived longer in the lower concentration of antibiotics than in the higher concentration and in the controls.

The original concentration of 50 mg/l. antibiotics appears to have some detrimental effect on the vitality of the copepods, which effect is reduced or eliminated by using a lower concentration. As to the effect of the penicillin medium on the bacteria, it should be kept in mind that an aqueous solution of penicillin is very unstable in sea-water. According to Korzybski and Kurylowicz[5], a penicillin solution at *p*H 5 at 0° C loses about half its activity within 8 days; in weak alkaline solutions, such as sea-water (*p*H 8), and also at higher temperatures, the process of inactivation requires considerably less time.

In order to evaluate under these conditions the effect of a penicillin solution on the bacterial growth in copepod cultures, an experiment was set up in which periodic counts of bacteria were made.

Three different media were tested: (*a*) penicillin medium (6·5 mg/l. penicillin + 37 mg/l. EDTA), (*b*) sterilized sea-water (autoclaved for 20 min at 110° C, + EDTA), and (*c*) control medium (natural sea-water + EDTA). Two Boveri capsules were filled with each medium and five

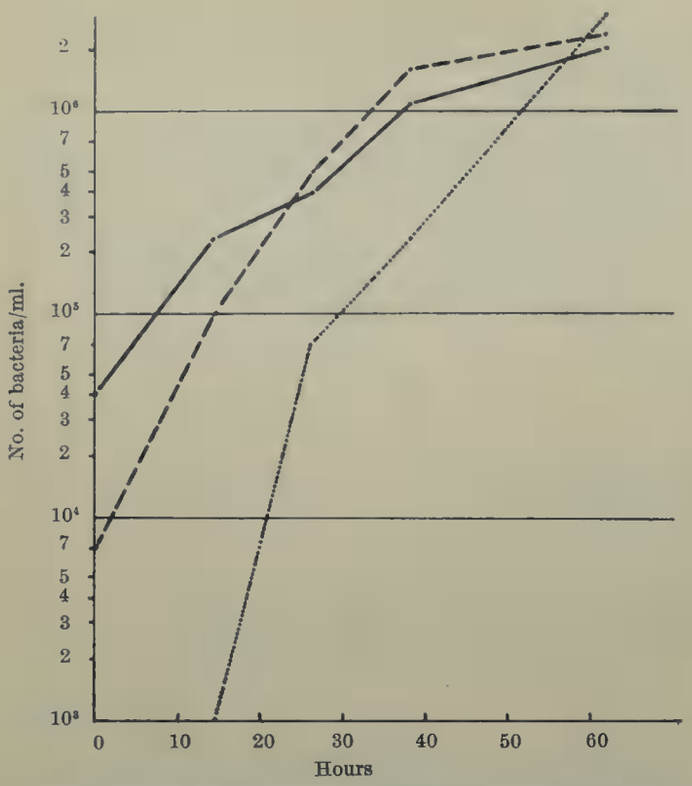

Fig. 1. Bacterial growth in copepod cultures, set up with different media: ——, natural sea-water; — — —, penicillin solution (6·5 mg/l.); . . . . , sterilized sea-water. EDTA was included in all media

copepodites of *Euterpina acutifrons* added per capsule. The serial dilution method was used for the bacteriological tests; the diluted samples were then poured on "peptone agar" plates (Oppenheimer and ZoBell[6]) and the developing colonies were counted in due course. Samples for the bacteriological counts were taken from the cultures at 0, 14, 26, 38 and 62 h. The results are given in Fig. 1.

The results confirm that an appreciable difference in the number of bacteria can be noted in the first 38 h only. At zero time, the number of bacteria in the penicillin medium was 18 per cent of that of the controls, at 14 h about 40 per cent; after 26 h the numbers of bacteria in the penicillin medium and in the controls were about equal. In the sterilized medium which had been contaminated with bacteria by the copepods, the bacteria needed 62 h to reach the level of the controls. A parallel experiment with mass cultures of zooplankton showed a longer survival time in the penicillin medium, but shorter in sterilized sea-water.

The fact that the penicillin medium has a bacteriostatic effect during a period of less than a day may well be of vital importance, because it is possible that the resistance of copepods against excessive bacterial growth is extremely low during the initial period in which they are probably in a weakened condition after having undergone the straining procedures of collection, the transportation to the laboratory and finally the transfer by means of a pipette into the culture vessels.

The shorter survival-time in sterilized sea-water may be due to chemical changes effected by the high autoclaving temperature.

On the basis of these findings, the lower penicillin concentration (6·5 mg/l.) was adopted for standard culture medium.

The first species of which we obtained nauplii in the laboratory was *Euterpina acutifrons* (Dana), a pelagic copepod of the sub-order Harpacticoida. The nauplii, hatched from the eggs of females caught near the coast on August 22, 1963, were transferred into a bottle with 3 l. of standard culture medium. Initially, the medium was changed weekly. The population established itself but remained at a very low density. When, however, the medium was renewed at monthly intervals, the population density increased rapidly. The population remained in the same vessel for one year. The masses of live and dead algae and detritus collecting at the bottom were never removed and did not have any obvious detrimental effect on the culture. In August 1964, the population was divided and distributed into several new containers.

The population was examined every 4 days over a period of several months. Young naupliar stages were always predominant, which suggests a rather high larval mortality.

Generally, the density was between 1,000 and 3,000 individuals per litre, with rather marked fluctuations. But high values such as the following have been observed: 500 adults, 2,000 copepodites, 2,500 nauplii IV–VI, 9,000 nauplii I–III. These numbers were calculated for 1 l. on the basis of replicate samples of 5 ml.

In order to determine the time required for the completion of one generation, the life history of single individuals was observed. Females with eggs were transferred from the laboratory population into Boveri capsules. On the following day, the nauplii which had hatched in the meantime were transferred singly into covered salt dishes containing about 3 ml. standard culture medium. Observations were made daily and the moulted skins were counted and removed. The mortality was high: only 22 out of 50 nauplii survived to the first copepodite stage and only two of those reached the adult stage. Another experiment was therefore set up with 50 nauplii of various stages taken directly from the laboratory population. Of these, 12 developed into adults. Table 1 gives a summary of the results, combining the two experiments. At 18° C the resulting average time for the completion of larval development (egg to adult) is between 17 and 18 days. This result is confirmed by another observation: in a population started anew with ten females bearing eggs, the first F_1-adults appeared after 14 days and the first F_2-nauplii after 17 days.

Table 1. Duration of Larval Stages in Single Cultures of *Euterpina acutifrons* at 18° C

			Length of larval period (days)						Overall
		N	0	1	2	3	4	5	av. (days)
Nauplius	I	57		44	12	1			1·3+0·5*
	II	38		23	14	1			1·4
	III	30	1	19	9	1			1·3
	IV	26		17	7	1		1	1·5
	V	16		18	8				1·5
	VI	23		22	1				1·0
Copepodite	I	23		11	10	2			1·6
	II	22		10	9	3			1·7
	III	22	1	8	6	6	1		1·9
	IV	20	1	7	11	1			1·6
	V	14		2	8	3	1		2·2
								Total	17·5

* To the mean time length of the naupliar stage I, 0·5 days were added, because at the time 0, the nauplii were between 0 and 24 h old.

This is not very different from records of other copepod species reared under similar conditions. The non-pelagic *Tigriopus brevicornis* needs 19·5 days on the average to grow from egg to adult (own observation), and *Acartia tonsa* has a generation time (egg to egg) of 21–30 days at 17° C (Zillioux and Wilson[1]).

If we assume a period of about 3 days for the time between emergence as an adult and hatching of its first eggs, a generation of *Euterpina*, under the conditions described, averages 20–21 days. On the basis of this average, we can assume that the population, which is now 18 months old, at present should contain individuals which belong to the 27th generation.

Originally it was presumed that pelagic populations of *Euterpina* might be less resistant to culture conditions than the coastal one which was used to set up our first laboratory culture. Therefore, on November 28, 1963, and January 21, 1964, two new cultures were started with *Euterpina* collected in the open sea.

The resulting laboratory population performed just as well as the original one derived from a coastal population; there appeared to be no difference in their adaptability to culture conditions. One of the populations was later transferred to a culture medium without antibiotics with no apparent detrimental effects. It seems safe to assume that antibiotics are necessary only for the initiation of a laboratory culture.

It should be noted that *Euterpina acutifrons* is closely related to the non-pelagic genus *Tigriopus* which lives in tide pools and is very easy to maintain in the laboratory. It might be interesting to carry out culture experiments with other pelagic Harpacticoida as well, in order to determine whether a relatively high adaptability to culture conditions is a general feature of this group.

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OPTICAL ROTATORY DISPERSION OF A CHLOROPLAST PREPARATION

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OPTICAL rotatory dispersion (ORD) was used more than a century ago by Biot, Pasteur and others for the study of molecular structure[1]. Its use on proteins dates back to the work of Hewitt[2] and Jessen-Hansen[3] in 1927. During the interim period, rotation measurements have been limited in the main to a few wave-lengths where convenient light sources could be found. However, in the past decade ORD has been undergoing a renaissance, largely as a result of new applications it has found in the study of molecular structure, and it is now considered one of the most powerful methods for studying conformation and conformational changes in biological macromolecules.

The most significant use of ORD in protein work is probably the estimation of helix content of a protein and the detection of helix-coil transition taking place in proteins during denaturation. Another interesting area of molecular conformation studied by ORD is that found by Blout and Stryer[4], who showed that Cotton effect can be induced in the absorption-band region of certain symmetrical dye molecules when they are complexed with a dissymmetric helical structure of a polypeptide. They also found that the Cotton effect reverses sign when the dye is bound to a polypeptide of an opposite helical sense. When the dye is bound to a polypeptide with random conformation, no Cotton effect is observed.

As ORD is sensitive to the conformation and conformational changes in proteins and to the effect of binding small molecules, we have investigated the rotatory properties of cholorophyll[5] and other pigments in solution as well as in the native environment of the chloroplast, to gain some insight about the nature of binding of these pigments and to correlate it with the photosynthetic activity of the chloroplasts. This article presents some preliminary observations made on a chloroplast preparation.

The problem of binding of chlorophyll to lipoprotein has been investigated by many workers in the past, mainly through such optical properties as absorption and emission. Although the state of chlorophyll *in vivo* is probably radically different from that of isolated molecules in solution, the absorption peaks of the bound pigment usually display only a slight shift as a result of binding[6]. Studies of optical anisotropy have usually led to the conclusion of a textural or form anisotropy in the chloroplasts[7–9]. More recently, polarized-absorption and -emission measurements[10] with *Euglena* chloroplasts and electric-dichroism measurements[11] with quantasomes have indicated an orientation of part of the chlorophyll molecules in the chloroplasts.

Chloroplasts are known to be sites of photosynthesis. As revealed by electron microscopy, chloroplasts consist of laminated systems embedded in a matrix, or stroma. The lamellae and stroma are surrounded by a double membrane. The dark layers in the laminated system are where the chlorophylls and other pigments are concentrated. Considered chemically, the laminated system is a gigantic organized complex of lipoprotein and chromophores. However, the so-called chlorophyll-lipoprotein has never been isolated from the chloroplasts in pure form. When chloroplasts are broken, the dark regions are released in particulate forms called 'grana'. The lamellae appear to be made of sub-units which were first observed by Frey-Wyssling and Steinmann[12], and have recently been given the name of 'quantasomes'[13]. The quantasomes are oblate spheroids 200 × 100 Å. It has been suggested that the quantasomes probably represent the smallest functional units capable of carrying out quantum conversion and electron transport.

The chloroplasts used in this work were prepared from spinach leaves. Quantasome aggregates were prepared according to the procedure described by Park and Pon[14], except that KCl was substituted for sucrose. Further rupture of the grana was achieved by sonication, and the lamellar fragments were fractionated and freed from stroma by differential centrifugation. ORD measurements were made in the Cary 'Model 60' spectropolarimeter, with cell compartments thermostatic at 24° C. Up to 600 mμ, the bandwidth was programmed at 3 mμ. Above 600 mμ, the bandwidth was manually set at 5 mμ to avoid excessive noise because of low light intensity.

ORD spectra of isolated chlorophylls *a* and *b* in diethyl ether over the visible and near ultra-violet regions have been reported recently[5]. Negative and positive Cotton effects were found to correspond to the major absorption bands in the red and blue regions, respectively. These Cotton effects indicate that the electronic transitions corresponding to the absorption bands are optically active. The Cotton-effect inflexion points correspond closely to the absorption peaks.

The ORD spectrum of the chloroplast suspension, shown in Fig. 1, shows considerably more complexity and intensity than that of the free chlorophylls. Cotton effects corresponding to almost all the absorption peaks of chlorophylls *a* and *b* are retained in the spectrum of the lipoprotein complex. For comparison, the ORD spectrum

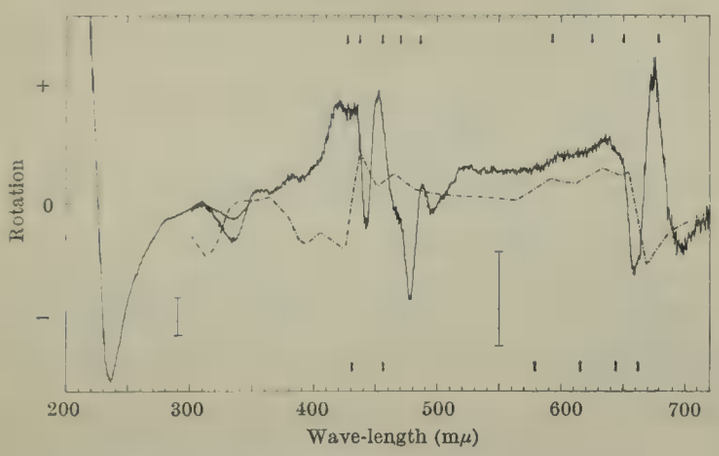

Fig. 1. ORD spectra of a suspension of the chloroplast lamellar fragments (recording trace) and an ether solution of chlorophylls *a* and *b* (dashed line). Both solutions contain the same amount of chlorophylls *a* (15 μg/ml.) and *b* (5 μg/ml.). Arrows at top indicate absorption peaks of the chloroplast suspension; arrows at bottom indicate absorption peaks of the chlorophyll solution in ether. The scale bars represent a rotation of 0·01°

of an ether solution containing equivalent amounts of isolated chlorophylls *a* and *b* is drawn in the same figure in dashed lines. The pigment absorption peaks of the chloroplast suspension and those of the free chlorophylls in ether in the visible region are indicated by arrows at the top and bottom of the spectra, respectively.

ORD measurements of mixtures of chlorophylls *a* and *b* at various proportions showed that the magnitude of rotation is apparently additive. Consequently, the Cotton effects of the chlorophyll mixture in the red region become less sharp. On the other hand, in the chloroplast suspension, the negative Cotton effects corresponding to chlorophyll *a* (678·5 mμ) and chlorophyll *b* (650 mμ) are completely resolved and their magnitude greatly enhanced. The positive Cotton effects corresponding to the two subsidiary bands of chlorophyll *a* (624 and 594 mμ) in the chloroplasts are also discernible.

The Cotton effects of the free chlorophyll solution in the Soret region are much better resolved, apparently because of a greater magnitude of the effects and a wider separation of the absorption peaks. However, in the chloroplasts, because of overlapping absorption by the chlorophylls and carotenoids, the assignments of the Cotton effects to the individual adsorption peaks between 400 and 500 mμ becomes difficult. This is especially true for the overlapping absorption between chlorophyll *b* (470 mμ) and the carotenoid band at 455 mμ and between chlorophyll *a* (437 mμ) and another carotenoid band at 428 mμ. Based on the known absorption-peak position of the carotenoids, the strong negative trough near 480 mμ was tentatively designated as a Cotton effect originating from the carotenoids.

A pronounced negative trough with peak wave-length at 235 mμ was observed in the chloroplast preparation. This is similar to the 233 mμ trough first observed by Simmons and Blout[15] in the protein sub-units of tobacco mosaic virus and later in various other polypeptides and proteins[1], and is believed to represent the n-π^* transition in the peptide chromophore. The magnitude of the 233 mμ trough has been used as an approximate measure of the *α*-helix content[16] in a polypeptide or protein. By the same procedure of calculation[16], and assuming the helical protein in the chloroplast lamellae to be a right-handed *α*-helix, the magnitude of the 'reduced mean residue rotation' corresponded to a helical content of the order of 15 per cent in the lamellar protein.

Our present knowledge about the nature of protein and lipids in the chloroplast lamellae is rather limited. Although the amino-acid composition of whole-leaf and chloroplastic proteins has been reported[17], the amino-acid sequence and protein conformation remain to be determined. Several earlier studies[9,18,19] reported the absence of ultra-violet dichroism in chloroplasts, and consequently Goedheer[9] and Ruch[18] proposed that the chlorophyll molecules were complexed with spherical protein molecules and that the pigment molecules were only slightly oriented. However, drawing analogies from the lamellar structure in the para-crystalline phase of poly-γ-benzyl-L-glutamate[19], it was suspected that chloroplast lamellae might also be composed of long parallel helices, with the pigment orientation governed by the available attachment sites. The low helical content found here was indeed contrary to expectation. However, the identification of the presence and the amount of α-helices in the chloroplast lamellae protein has lent a definitive character to the structural protein in the chloroplasts and furnished a conformational basis for any orientational effect on the pigments by the protein. There is no evidence that there might be a mixture of right- and left-handed helices present in the chloroplast lamellae. However, such a possibility should not be completely ruled out without further investigation.

The enhancement of the intrinsic Cotton effects of chlorophylls and carotenoids complexed with the lipoprotein suggests a strong interaction either between the pigment molecules themselves or between the pigment molecules and the attached macromolecules. The enhancement of rotation in the carotenoid absorption region appears to be especially strong. Although the xanthophylls present in spinach (the oxygen-containing derivatives of α-carotene) are known to be optically active, examination of a hexane extract containing ten times the combined carotenoid concentration of the chloroplast sample yielded only negligible rotation, mostly buried in the noise. The strong rotation in the carotenoid absorption region suggests a possibility of an orientational effect by the lipid matrix, resulting in either an induced Cotton effect in the carotenoids including the optically inactive β-carotene or an enhancement of rotation of the intrinsic optically active xanthophylls. Presumably the lipid matrix could also exert an orientational effect on the chlorophylls through the phytol chain. A definitive clarification to these suggestions obviously requires further investigation. ORD measurements appear to offer new avenues for such studies.

After the completion of this work, I learned the results of a similar study on quantasomes by K. Sauer[20]. It has been found that isolated chlorophyll *a* at a high concentration in carbon tetrachloride exhibits profound changes in its ORD spectrum. Sauer attributed such enhancements to chlorophyll aggregation and felt that a similar pigment–pigment interaction could also account for the enhanced ORD spectrum observed in quantasomes.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Rotational Period of the Planet Mercury

In a recent communication by S. J. Peale and T. Gold[1] the rotational period of Mercury, determined from radar Doppler-spread measurements to be 59 ± 5 days[2], has been explained in terms of a solar tidal torque effect, taking into account the large eccentricity of Mercury's orbit, and the $1/r^6$ dependence of the tidal friction (r being the Sun–planet distance). They conclude from a very brief discussion that after slowing down from a higher direct angular velocity, the planet will have a final period of rotation between 56 and 88 days, depending on the assumed form of the dissipation function. However, from their discussion it is by no means clear why permanent deformations would imply a period of 88 days as a final rotation state after a slowing-down process. A very nearly uniform rotational motion of 58·65 sidereal-day period, that is 2/3 of the orbital period, may indeed be a stable periodic solution. This rotational motion could have the axis of minimum moments of inertia nearly aligned with the Sun–Mercury radius vector at every perihelion passage. The orbital angular velocity at perihelion ($2\pi/56{\cdot}6$ days) is close to $2\pi/58{\cdot}65$ days, leading to an approximate alignment of the axis of minimum moment of inertia with the radius vector in an arc around perihelion where the interaction is strongest. The axial asymmetry of Mercury's inertia ellipsoid may result in a torque that counterbalances the tidal torque, giving a stable motion with this orientation and with a period two-thirds of the orbital period. It would therefore be possible for Mercury to have a higher permanent rigidity than that permitted by Peale and Gold.

In discussion with I. I. Shapiro[3], we concluded that the actual rotational motion may have evolved via a speeding-up process from a lower angular velocity or possibly from a retrograde motion. We would point out that a 58·65-day period, precisely because it is 2/3 of the orbital period, fits some of the old optical observations as well as the recent radar measurements.

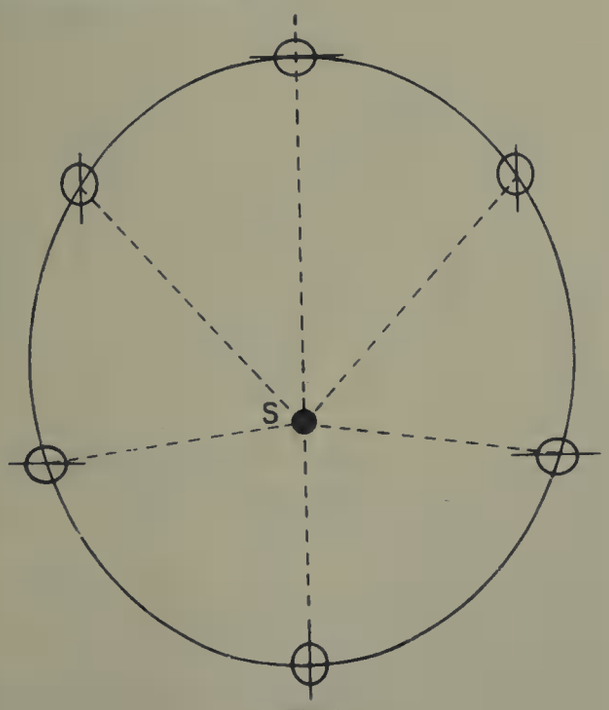

Fig. 1

In Fig. 1 a rough planar sketch is shown of the orientation of Mercury's axis of minimum moment of inertia, at different points along its orbit, given that the rotational period is two-thirds of the orbital period and that this axis is aligned with the Sun–planet vector at perihelion.

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ASTRONOMY

Filamentary Nebulosity in the Vicinity of the Cetus Arc

Forming large diameter circles across the sky are three ridges of enhanced radio emission. They are the North Polar Spur[1], 111° in diameter, the Cetus Arc[2], 91° diameter, and a third, unnamed, 60° diameter loop centred on the northern celestial pole[3].

The radio continuum emission from these features is identical to that from the old supernova remnants. However, the nature of the 60° diameter loop is complicated by its correlation with twelve high-velocity neutral hydrogen clouds[4]. It has therefore been suggested that two, if not the third, of these objects are the remnants of nearby supernova explosions.

The principal difficulty to the acceptance of this theory has been the lack of detection of any optical emission regions coincident with these loops[5,6]. All the accepted supernova remnants have characteristic filamentary nebulosity bounding their radio regions.

Such nebulosity has recently been searched for, on these three radio sources, in a photographic programme carried out at the high-altitude observatories of Jungfraujoch and Pic du Midi. Photographs were taken with small, high-speed camera and filter combinations which had low detection limits for emission nebulosity.

A further effective method of detecting faint, large-scale nebulosity was also used on this problem. In this, Palomar Sky Survey plates were copied at reduced scale and very high contrast. These were then compiled to form mosaics across the radio features. Faint density changes that were continuous through adjacent plates were considered to be caused by nebulosity.

The results produced by this work are as follows: (*a*) No filamentary nebulosity was detected on the North Polar Spur. However, a very diffuse optical emission region was shown to be present over this area. As yet it is impossible to say whether or not this is associated with the radio feature. (*b*) No filamentary nebulosity has been found on the 60° diameter loop. A search for diffuse nebulosity is now being undertaken. (*c*) It was on the Cetus Arc that the most interesting nebulosity was discovered. This has been sketched against the 237-Mc/s radio contours and presented in Fig. 1. The most striking single feature of this optical emission zone is the huge arc of nebulosity which bounds it. This is of filamentary microstructure and has so far been traced for a length of 28°. Within this arc, towards the Cetus Arc radio ridge, there is a zone of diffuse elongated filaments. These merge with a structureless optical emission region as the radio

ridge is crossed. This very diffuse nebulosity has not been sketched, as its extent is ill-defined.

The nebulous arc from $\alpha = 4$ h $\delta = +13°$ to $\alpha = 3$ h 40 m $\delta = -15°$ is comparable, in position relative to the radio feature and in structure, to the optical emission regions associated with the Cygnus Loop and *IC*443 (refs. 7 and 8), both of which are accepted supernova remnants. However, a further experiment is required to test conclusively the suggestions that it is associated with the Cetus Arc and that these are the optical and radio remnants of the same supernova explosion; that is, direct comparison of the Cetus Arc with the Cygnus Loop suggests that an angular movement of the Cetus Arc nebulosity can be expected of 1″ to 3″ per annum. This motion should be occurring away from the centre of the circle described by the Cetus Arc if this comparison is correct. In this case, during the ten years since the Palomar Plates were taken, where this nebulosity appears, the total angular displacement should amount to 10″–30″. Such a displacement could be easily detected, for the fine filaments of the nebulosity have similar angular dimensions.

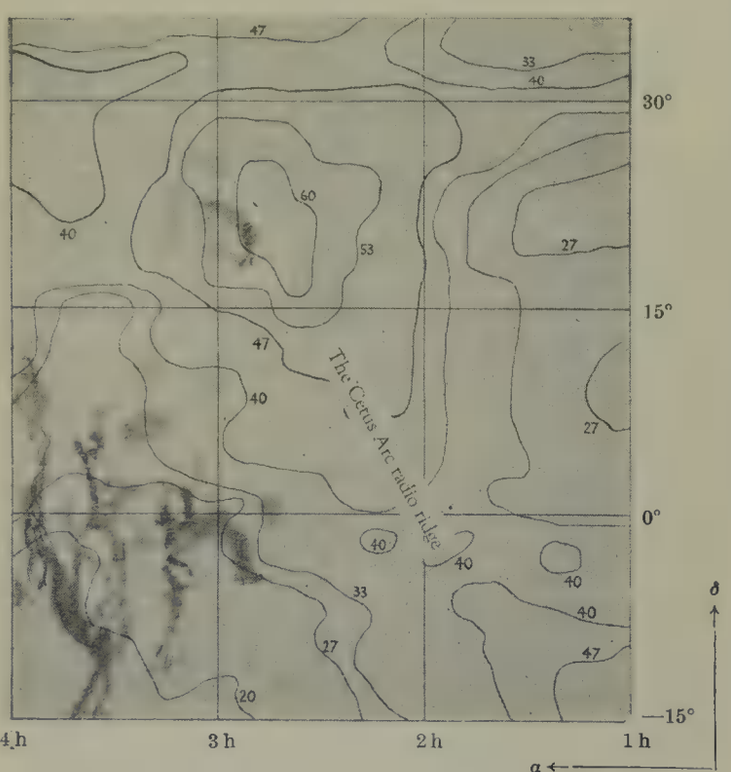

Fig. 1. The nebulosity is shown as a heavily shaded area. The continuous lines are the 237-Mc/s radio contours[2]. Their brightnesses are given in degrees K

This discovery of filamentary nebulosity bounding the Cetus Arc has strengthened the theory that this object and the other two similar radio features are all supernova remnants. However, the experiment to measure its rate of angular movement could, if positive results emerge, decide this issue conclusively.

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PHYSICS

Carathéodory's Principle and the Kelvin Statement of the Second Law

The second law of thermodynamics has been stated in various ways. The two forms with which this communication is concerned are: (*a*) Kelvin's principle that it is impossible to transform an amount of heat completely into work in a cyclic process in the absence of other effects. (*b*) Carathéodory's principle that all points A in thermodynamic phase space are i-points[1]; that is, in every neighbourhood of every point A there are points adiabatically inaccessible from A. It has already been shown that (*a*) implies (*b*)[2] and, in this communication, it is proposed to explain in what sense (*b*) implies (*a*).

The n-dimensional thermodynamic phase space may be thought of as having $(n-1)$ deformation co-ordinates $v_1, v_2, \ldots, v_{n-1}$ and one thermal co-ordinate, which will be taken to be the internal energy, U. By assuming the validity of Carathéodory's principle and the existence of adequate continuity conditions, it may be shown[3] that all points in this space which are accessible from some given point P by quasi-static adiabatic processes lie on a single surface, and that P itself must lie on this surface. The argument may be repeated for different initial states P and the thermodynamic phase space is found to be decomposed into a family of non-intersecting surfaces. These are surfaces of constant entropy. Clearly, points lying in different level surfaces cannot be linked by quasi-static adiabatic processes.

In thermodynamic phase space, consider two points A and B ($U_B < U_A$) lying on a line of constant deformation $(v_1, v_2, \ldots, v_{n-1})$. Since each line of constant deformation cuts each level surface in one point, the two points A and B lie in different level surfaces and, hence, cannot be linked by quasi-static adiabatic processes but, either: (i) A is accessible from B by a non-static adiabatic process, or (ii) B is accessible from A by a non-static adiabatic process, but not both. This result is a consequence of Carathéodory's principle and continuity assumptions which represent the basic premises of this communication.

Suppose (i) holds. The system may be taken from B to A by a non-static adiabatic process and the increase in internal energy is due to mechanical work W done on the system. With deformation co-ordinates fixed, the system may be returned from A to B. With the v_i constant, no work is done and the decrease in internal energy is due to heat $Q = U_A - U_B > 0$ having been removed. In this cycle, due to the conservation of energy, an amount of work is seen to be completely converted into heat.

Suppose (ii) holds. With deformation co-ordinates fixed, the system may be taken from B to A. With the v_i constant, no work is done and the increase in internal energy is due to heat $Q = U_A - U_B > 0$ having been supplied. The system may then be returned from A to B by a non-static adiabatic process and the decrease in internal energy is due to mechanical work W done by the system. Due to conservation of energy, it is seen that an amount of heat is completely converted into work in this cycle.

It follows, therefore, that for the cycles considered, either: (1) work cannot be completely converted into heat, or (2) heat cannot be completely converted into work. This multiplicity of possibilities is characteristic of conclusions based on Carathéodory's principle. Unless supplemented by additional assumptions, it leads to four possible types of thermodynamics, all similar in logical stucture but distinct in detail[1].

To obtain Kelvin's principle, not only must adequate continuity assumptions be made but it must be assumed that any given cycle can be decomposed into a number of cycles of the type considered here and, in addition, that (2) rather than (1) holds. It is only in this sense that Kelvin's principle may be deduced from Carathéodory's

principle. Of these additional assumptions, the second is seen to be valid in the usual type of thermodynamics (referred to as type I in ref. 1), in which the absolute temperature is positive and heat tends to flow from high to low absolute temperatures. It is the absence of these additional assumptions which gives the converse argument[2], the deduction of (*b*) from (*a*), an attractive simplicity.

The form of Kelvin's principle applicable for negative temperatures states that in a cyclic process, in the absence of other effects, either heat cannot be completely converted into work or work cannot be completely converted into heat[4]. It may be noticed that, apart from the basic premises, only the assumption that any given cycle can be decomposed into cycles of the type considered here is needed to deduce this modified form of Kelvin's principle. It is only if one wants to go further and derive the conventional form (*a*) of the Kelvin principle that the additional assumption, that (2) rather than (1) holds, must be made.

I thank Prof. P. T. Landsberg for his advice and also for the preprint of a paper by Sears[5], in which the converse of the argument in ref. 2 is discussed in an alternative way. This work was supported by a scholarship awarded by the Glamorgan County Council.

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Ionizing Radiation : a Potential Lightning Hazard?

Benjamin Franklin and Michael Faraday were until recently the only major contributors to the problem of preventing lightning damage to man-made structures. The 'Franklin Rod' and the 'Faraday Cage' systems both afford a more conductive path to earth for the lightning strike than that supplied by the structure they are intended to protect.

The electric charges generated in a thunder cloud are not distributed evenly throughout the cloud but form regions of higher intensity of electrostatic charge. It is from these regions to a region of high induced intensity of charge on the earth that the lightning discharge will tend to occur. This is because some points in this region of high intensity on the ground will liberate a stream of positive ions (known as the 'point discharge current'), resulting in a region of ionized and therefore more conductive air extending above this point. The most highly conductive path from cloud to earth is searched out by an initial low-voltage discharge; the so-called 'pilot streamer', which is followed by a second, fairly slow-moving discharge, the 'stepped leader'. This prepares a well-ionized path for the one or more main strikes which follow; these usually involve potentials of between 10^8 and 10^9 V and currents in the range of 20 to 90 thousand amperes.

The probability of any point being selected as the site of a lightning strike depends on many factors, but essentially these can be reduced to two: (1) The height of this point above other prominences in the region; (2) the magnitude of the point discharge current and thus the conductivity of the air above the point. While these factors determine the point in a region which will be struck, other factors, geographical, meteorological, etc., make some regions more prone to lightning strikes than others.

In the protection of man-made structures from lightning damage, the most important factor is usually considered to be the height of the building. In Great Britain it is found that churches are struck fifteen times more frequently than private dwelling-houses[1]. The estimated frequency of lightning strokes to earth in England is about six per square mile per annum[2] of which approximately one quarter cause damage to persons or structures.

In recent years a new type of lightning protection device has been developed, and the fact that legislation governing its use has been passed under the 1960 Radioactive Substances Act would suggest that it is becoming widely used. The operation of this device is based on the ability of radioactive material to ionize the air surrounding it, the extent and degree of ionization depending on the activity and nature of the source. Suitably encapsulated radioactive sources are placed slightly higher than the roof of the building to be protected and are carefully earthed in the same way as the traditional devices. Any lightning strike entering the hemisphere of effective ionization caused by the source should be directed along the increasingly well-ionized path, strike the source itself, and be conducted harmlessly to earth. This hemisphere of effective ionization is arranged to cover the entire roof area of the building, and thus the building itself should never be struck as there will always be a more conductive path leading to the well-earthed source.

A commercially available type designed to afford a hemisphere of protection of radius 100 m contains 708-μc. radium-226. The radiation dose rate at 100 m from a radium source of this size will not be greater than 0·00006 mr./h, and may well be less, due to absorption in the encapsulating material and also self-absorption in the source itself. This extremely small dose rate is considered to produce sufficient ionization to make this path to earth the most likely to be chosen by the lightning, and experiments carried out by the manufacturers are claimed to substantiate the effectiveness of this method. If this is so, the potential hazard due to the lightning-attractive properties of radioactive sources used for medical and industrial purposes would not appear to be widely recognized. This could result in a structure housing a source of ionizing radiation, and therefore presumably a particularly attractive site for a lightning strike, being totally unprotected against this occurrence.

Possibly persons using radioactive material for medical and industrial purposes are not aware of the claims made for the lightning-attractive powers of sources giving small radiation dose rates, or perhaps this aspect of a source used primarily for a totally different purpose has not been considered.

It is of interest to consider the case of a hospital in which kilocurie sources of γ-emitting radioisotope, for example, cobalt-60, are used for radiotherapeutic purposes. Therapy units containing these large sources are commonly installed in single-storey buildings, which are normally not fitted with any earthing system for lightning. The main radiation beam from a rotational therapy unit is frequently directed towards the roof of the therapy room for a percentage of the total treatment time. The attenuation produced by the roof is often minimal (for example, a roof light of ordinary glass) as the radiation scattered from the emergent beam is not considered to constitute a hazard to personnel at ground-level.

A typical 3,000-c. cobalt-60 source gives a dose rate of approximately 125 r./min at 65 cm from the source. Allowing for absorption in the patient's body through which the beam passes, a rough calculation reveals that a dose rate of 6×10^{-5} mr./h (that is, the dose rate produced by the lightning protective device at its effective range) will still occur at a height of 62 miles. When the radiation beam is not directed upwards, only scattered radiation emerges through the roof. If one assumes that the intensity of this

scatter is only a ten-thousandth of that of the main beam, this still corresponds to a height of more than half a mile before the dose rate falls below that considered effective to attract the lightning strike to the source.

Many industries use radioactive sources giving local dose rates far in excess of 6×10^{-5} mr./h, and indeed I am aware of such a source used as a density gauge mounted on the upper section of an outdoor chemical plant synthesizing highly inflammable and explosive liquids.

In view of the foregoing, one would expect frequent reports of radiotherapy centres, industrial radiography departments, etc., being damaged by lightning; in fact, the search for reports of such occurrences has revealed only a vague rumour of this having happened once to a radiotherapy centre in the United States.

Must one therefore conclude that: (*a*) small sources of ionizing radiation are not effective as lightning attractors? or, (*b*) radiotherapy centres, etc., rarely operate during thunderstorms, and have, so far, been very fortunate?

This is a question to which some answer must be found, as at present neither of the two logical alternatives is satisfactory: either buildings 'protected' by these radioactive devices are in fact not actually protected and may well suffer lightning damage; or, sooner or later, a radiation beam will attract a lightning strike to the source of a tele-isotope unit and, unless this is as effectively earthed as a proper lightning conductor, the effects could be catastrophic. Energies of more than 5×10^{10} watts are usually involved and, apart from structural damage and the possible rupture of the sealed radioactive source, with resultant contamination, one hesitates to contemplate the fate of the unfortunate patient undergoing treatment.

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GEOLOGY

Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire

During recent investigation of some welded tuffs in the Ordovician rocks of eastern Murrisk, County Mayo, lenses of sillar, non-welded material were encountered at the base of the tuff-horizons. Similar layers had previously been reported by Stanton[1] and Dewey[2] during investigations farther west. Stanton[1] interpreted the variation in thickness of the sillar material as due to differences in the depth of sea-water present before the tuff built up to sea-level, prior to it becoming a truly welded tuff. The entire process was regarded as a 'continuous whole'.

A letter written by the British Consul in St. Pierre on May 3, 1902, some five days before the Peléan eruption which destroyed the town and its inhabitants, has been brought to my attention by Mr. R. A. Japp, of Dundee, together with samples of the early ashes produced during the eruption. With permission, an extract from this letter is relevant:

"I wrote you about the volcano smoking last week. Yesterday afternoon it began to throw out enormous clouds of steam and black smoke from a different place.

"About one this morning I was awakened by a roaring noise, something like thunder and lightning. . . . The houses and street were covered with dust . . . white, as if there had been a snow-storm.

"The dust continued to fall until there was a layer of ¼ inch, and is still falling a little. It is all very fine (I enclose sample), and as we have no glass, but only 'jalousie' windows, it drives in and penetrates everywhere . . .

"The volcano is 4 miles from here. A river running from it was swollen with dirty water and mud, and made a great stink . . . Today the smoke and dust have spread—some of the latter having gone beyond Fort de France—20 miles away. I believe there will be no danger . . ."

The sample referred to has now been examined and found to consist principally of a very fine siliceous ash, in which occur broken and angular fragments (up to 0·05 mm in diameter) of crystals of quartz and labradorite feldspar. The labradorite (An_{55-70}) shows both multiple albite twinning and complex oscillatory zoning in which outer zones have sometimes been partly torn from the cores of the crystals. Some magnetite is present, but there are no recognizable shards in this very fine-grained, yellow-green tuff.

This account is of two-fold significance. First, it directs attention to the importance of the preliminary phases of eruption which precede the extrusion and accumulation of Peléan tuffs. Secondly, it indicates that the sillar bases to welded tuffs need not be strictly contemporaneous with the overlying welded material. MacGregor[3] and others have pointed out that the terms 'Peléan' and 'welded' tuffs are not necessarily synonymous.

The observation that the nearby rivers were swollen with debris from the early fall-out of ash shows that the loose ashes were rapidly transported to the sea. In areas where shallow water bordered the land, this fine ash would have been rapidly redistributed and deposited on the sea floor. Protracted activity of this type enables appreciable accumulations to develop before the extrusion of the main mass of pyroclastic material. The preliminary phases of such eruptions, therefore, may be responsible for the shallowing of the sea, so that the hot ash falling into the shallow water rapidly builds above water-level, so permitting welding to occur above the non-welded, non-contemporaneous base.

The welded tuffs of western Mayo show non-welded sillar bases in various places. In western Murrisk both Stanton[1] and Dewey[2] have recorded sillar layers in *MT*1 and *MT*2. In eastern Murrisk neither of these tuff layers has a continuous sillar base. Thin lenses are present on *MT*1, but the base of *MT*5 has a 4-ft.-thick layer of sillar material in the cliffs to the west of Lough Glenawough. No sillar material is present east of the lake.

The lowermost part of the sillar layer is almost devoid of shards. In places it consists of very fine dust with a few grains of quartz, which show uniform extinction. In the upper part of the sillar layer angular fragments of untwinned plagioclase An_{25-40} are abundant in the tuff, with less frequent angular quartz grains, a few of which show the deep embayments so common in the quartz grains of the overlying, welded tuffs. Virtually all the feldspar fragments are deeply altered to sericitic material, with the result that they are distinguished with difficulty from the intratelluric material, in which minute undeformed shards are present.

With the change from non-welded to welded tuffs there is an increase in the size of the shards, from 0·01 to 0·04 mm to 0·05 to 0·20 mm. There is an accompanying change in the proportion of included lithic fragments (0·2–3·5 per cent). These two factors may result from changes in the strength of the forces active at different stages of the eruption. Large shards are to be expected from the fully frothing pyroclastic flows, whereas smaller ones result from quieter, less spectacular eruptions of the early phases.

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Origin of Fossil 'Zoophycos'

In the Czechoslovak Carpathians I have found imprints, described in the literature from many localities throughout the world as *Zoophycos*, *Taonurus*, *Spirophyton*, etc. The occurrence of these imprints in numerous localities in the Czechoslovak Carpathians is closely related to fine-grained sandstone strata, and sometimes to the marlstones (Cretaceous, Palaeogene). After a detailed investigation I concluded that these imprints were formed by abandoned prostomial parts (gill organs) of sedentary marine worms (Annelida, Polychaeta) from the family Sabellidae[1]. The imprints are closely similar to the recent genus *Spirographis Viviani* 1805. As in that genus, the gill rays of the fossil worms are spirally wound around an asymmetrical gill lobe either to the left or to the right side, in the ratio of about 1:1. In sedimentary rocks the gill organs are either perpendicular to the bedding plane or they form flat coinciding accumulations.

The gill organs (prostomium) which are the forepart of the body are easily abandoned by the worms when they are in danger[2]. Abandoned gill organs were formerly considered by palaeontologists as unknown, extinct plants, most probably algae or as a breeding place of marine animals, so that these fossils were regarded for a long time as problematical in origin. They are common in Africa[3,4], America[5,6], France[7], Germany[8], England[9], Switzerland[7], Belgium[8,9], Luxembourg[8], Italy[10], Austria[11], Poland[12], Czechoslovakia[1], and the U.S.S.R.[13], and their occurrence may be recorded anywhere in shallow-water marine sediments all over the world.

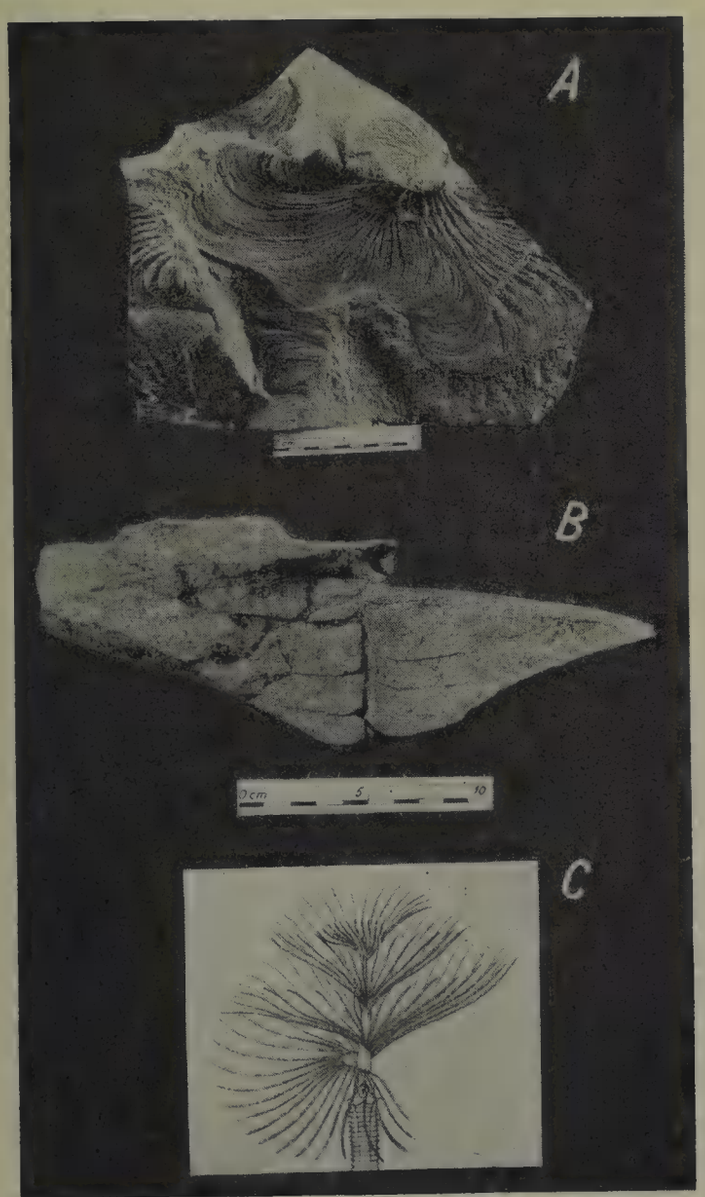

Fig. 1. *A*, *Zoophycos*; the imprint of the lower floor of branchial filaments when observed from above. The Carpathian flysch, Eocene, sample No. 33120*B*–5/1, photo Z. Šerebl. *B*, *Zoophycos*; the imprint of prostomial lobe with branchial filaments when observed from one side (longitudinal section). The Carpathian flysch, Palaeocene, sample No. 3497*C*–1/1, photo Z. Šerebl. *C*, *Spirographis spallanzanii Viviani*; the prostomial lobe with branchial filaments when observed from one side (A. Lameere[14])

Fossil worms lived in a siphuncle as sedentary animals in colonies on the bottom of a shallow sea and, like recent forms, they fed on fine detritus and micro-organisms sinking downwards to the bottom. They received the food by means of their gill organs with variously adapted gill rays. On account of the different shape of the gill rays in fossils from the Flysch of the Czechoslovak Carpathians I proposed several genera. In that area, where these fossils occur in the rocks, a very rapid sedimentation may be supposed.

Due to their systematic classification in the animal kingdom they have become the most common fossils in the stratigraphic as well as in the topographic sense and they ought to have been referred to the zoological system long ago.

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METALLURGY

Dispersion-softened Zinc Alloys

A number of zinc-based alloys containing equiaxed particles of various stable and insoluble hard second phases were prepared as part of an investigation into the high-temperature compression creep characteristics of dispersion-hardened materials[1]. It was found that the dispersed particles produced a substantial strengthening effect at temperatures above 0·60 of the absolute melting temperature of zinc. Room-temperature tensile tests were made in order to measure the expected loss in ductility of zinc caused by the hard particles. It was surprising to find, therefore, that most of the dispersed particles reduced the strength and increased the ductility of zinc. These results are briefly reviewed here.

The alloys were prepared by methods of powder metallurgy in order to produce uniform dispersions of ZnO, αAl_2O_3, carbon black, and tungsten in a void-free zinc matrix. The particle size of these spheroidal dispersoids was typically 0·1–0·6μ. Two concentrations of second-phase particles were studied: 9 and 15 volume per cent. The composites were obtained in the form of 0·375-in.-diameter extruded rods. Specimens were annealed before testing at 385° C for 24 h. Pure zinc extrusions were also prepared from the same lot of powder as the composites and these were used to establish a basis for comparison.

Typical true stress versus true strain curves are presented in Fig. 1 for a zinc–tungsten alloy and also for a pure-zinc specimen. There are three characteristics to be noted from these curves and from the results in general: (1) The dispersed particles of tungsten actually weaken the metal. It can be seen that both the stress at the onset of plastic flow and the ultimate strength were lower for the composite than for the pure zinc. (2) The particles produced strain softening in zinc. That is, after a period of work-hardening, the effective stress required to deform

further the composite decreased with increasing strain. (3) The composites were considerably more ductile than is pure zinc. These three effects were most pronounced in the zinc–tungsten alloys, but they were also observed in each of the other composites containing spheroidal dispersed particles except for the alloys of zinc containing carbon. The influence of carbon was to embrittle as well as to weaken zinc (for example, 1–5 per cent reduction in area for Zn–C versus 14–23 per cent in pure zinc and 50–93 per cent for Zn–W). Some materials containing zinc oxide in platelet rather than in spheroidal form behaved in a more conventional manner. That is, they were stronger but slightly less ductile than pure zinc.

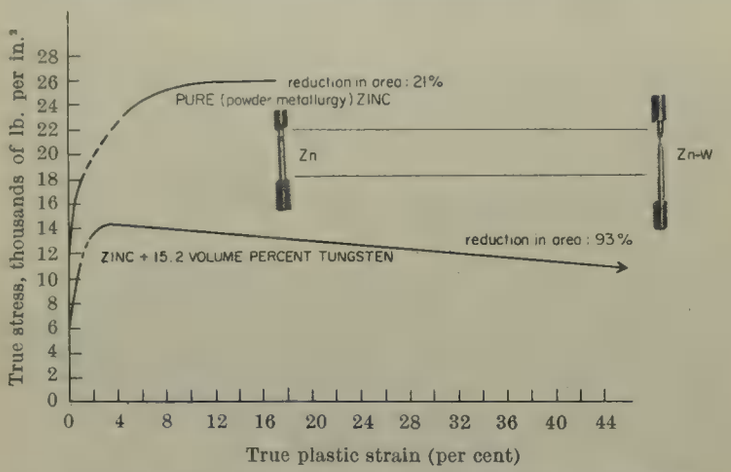

Fig. 1. Tensile behaviour of a zinc–tungsten dispersion-softened alloy compared to pure zinc. True stress-true strain curves obtained at 26° C and at a strain rate of 2 h^{-1}

An examination of the slip bands produced in deformed specimens of pure zinc showed that the bands were coarse and widely spaced. Very fine slip lines were observed in the deformed zinc–tungsten alloy and electron microscopy was required to resolve them. Duplex slip, which manifested itself by intersecting slip lines, was common in the composites.

We believe that the fine slip and the duplex slip account for the reduced strength, work softening, and improved ductility that the alloys exhibited. Duplex slip can lead to the intersection of dislocations resulting in the formation of many jogged screw dislocations. Intersection is minimized when slip is restricted to the basal plane. The predominance of duplex slip in the composite alloys suggests that there were many more intersection-induced dislocation jogs in the alloys than in the pure zinc. The motion of jogged screw dislocations will produce an excess vacancy concentration[2,3] which should aid the climb of dislocations over the dispersed particles. Thus the composite is strain softened and its ductility is enhanced. In addition, the ductility of the composite is high since the presence of fine and duplex slip will give a more uniform distribution of strain than the presence of coarse slip bands as was observed in zinc. It is likely that the fine slip and duplex slip observed are promoted, and indeed may be caused, by the microscopically non-uniform distribution of stresses that must exist in the alloys. This stress field heterogeneity may be caused by stress concentrations near each particle in a specimen subjected to an applied load and it may also be the result of the thermal expansion misfit between particles and matrix that develops when the alloy is cooled from the extrusion or annealing temperature. These internal stresses in the composite can lead to a low yield stress and a low ultimate strength in comparison to pure zinc.

Polycrystalline beryllium and magnesium are similar to zinc in that they exhibit limited ductility at low temperatures. This low ductility is generally attributed to the limited slip systems available in these hexagonal close-packed metals. It is possible that the addition of fine particles of selected high-melting second phases to beryllium or magnesium may improve their ductility as was observed for zinc.

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CHEMISTRY

Structure of a Diolefine Complex of Platinum(II) $C_{10}H_{14}O_3PtCl_2$

The nuclear magnetic resonance and infra-red spectra[1,2] of potassium *tris* acetylacetonatoplatinum(II) suggest that the acetylacetone anions form a square planar arrangement around the platinum with one conventional oxygen-bonded acetylacetone chelate and two γ-carbon bonded ligands. The complex reacts with hydrochloric and hydrobromic acids to give greenish-yellow complexes of composition $C_{10}H_{14}O_3PtX_2$ (X = Cl, Br) which are monomeric in chloroform and non-electrolytes in nitromethane. In contrast to the 'acid' derivatives[3] of the complex $K[Pt(Acac)_2X]$, these complexes are stable in solution for several days.

The nuclear magnetic resonance spectra of $C_{10}H_{14}O_3PtCl_2$ and $C_{10}H_{14}O_3PtBr_2$ indicate a symmetrical structure with two sets of pairs of methyl protons at 8·3τ and 7·8τ and two olefinic protons at 5·5τ; the methyl protons have $^{195}Pt-H$ coupling constants of 3 c/s (8·3τ) and 34 c/s (7·8τ), the protons at 5·5τ having a coupling constant of 84 c/s. These coupling constants are consistent with the co-ordination of the platinum by olefinic groups with one pair of methyl groups directly bonded to the olefine and one set separated by a number of intermediate atoms. The infra-red spectrum is also consistent with the presence of a co-ordinated olefine grouping ($\nu_{C=C}$ = 1,495 cm^{-1}) and the absence of a co-ordinated acetylacetone ligand. A characteristic absorption at 1,236 cm^{-1} implies the presence of an unsaturated ether grouping, the two platinum-chlorine stretching frequencies at 325 and 335 cm^{-1} requiring a *cis*- arrangement of the chlorines in the complex. The identity of the infra-red spectra of the chloride and bromide spectra above 400 cm^{-1} shows that there are no direct carbon–halogen bonds in the complex.

β-Diketones are known to condense with elimination of water to give further unsaturated systems; the presence of only three oxygen atoms in the complex and the absence of an O–H group absorption in the infra-red and nuclear magnetic resonance spectra suggest that such a condensation has taken place in the reaction of $K[Pt(Acac)_3]$ with acids. The structure of the condensation product, which was suggested by the spectral data, has now been confirmed in detail by a single crystal X-ray diffraction analysis. The X-ray data collected, by courtesy of Dr. P. B. Braun, on a 'Pailred' automatic diffractometer, have been converged to a present reliability index of 0·060, the overall stereochemistry of the complex being shown in Fig. 1.

The co-ordination of the platinum is approximately square planar with two chlorines, in a *cis* configuration, at a mean distance of 2·31 Å (σ 0·005 Å), and two donor olefinic bonds of mean length 1·45 Å (σ 0·03 Å). The bonding of each olefinic group is asymmetric, the mean Pt–C distances being 2·10 Å and 2·33 Å (σ 0·02 Å). The

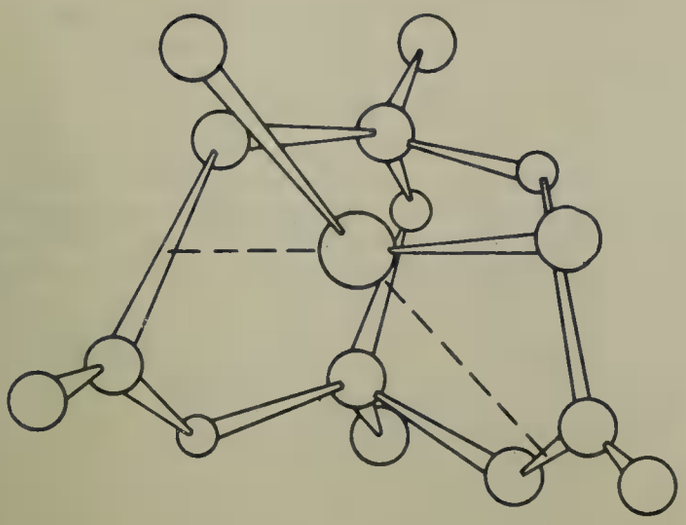

Fig. 1

intramolecular platinum–oxygen distances range upwards from 2·89 Å, more than 1 Å greater than is found in the example of the oxygen-chelated acetylacetonate anion in K Pt(Acac)$_2$Cl [1]. The mean lengths of the bridging and remaining C—O bonds are 1·44 and 1·40 Å respectively (each with σ 0·03 Å).

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Ultra-purification by Separation of Aerosol Particles

Purification methods which treat the concentration of a given impurity as a continuous quantity become ineffective when only a discontinuous distribution of that impurity is left in a material. Further purification is feasible on a dispersion principle which makes use of this discontinuity, and by which liquids have been observed free from particles at temperatures too low to cause the latter to be melted or dissolved.

A bulk sample of liquid, which after thorough pre-purification still contains a number of particles, is partitioned into a larger number of droplets which are usually suspended in a particle-free fluid. In the resulting dispersion, the particles are confined to a fraction of the droplets and thus isolated from the rest of them[1,2].

Liquid dispersions of metals[1–4], water[5–7], organic compounds[8,9], and alkali halides[10], of uniform droplet size, were subjected to under-cooling. Meanwhile, the droplets were observed either collectively or singly; their solidification was detected by inoculation of under-cooled bulk liquid[5], by optical effects, for example, recalescence flashes[3], twinkling[8,10], or X-ray diffraction[4], and by other techniques. A portion of the droplets did not freeze until, far below the melting point, the critical under-cooling temperature had been reached. There is evidence that these droplets crystallized by homogeneous nucleation and therefore were free from heterogeneous impurities. Homogeneous impurities did not alter very much the critical under-cooling temperature[8], unless, as in emulsions, they were occluded in the particles[9].

The isolation of particle-free liquids was always incidental to the investigation of other topics and the possibility of using it to produce these liquids in appreciable quantity does not seem to have been realized. For example, a drop (2 mm diameter) of particle-free water, stored at the interface between two immiscible liquids, was instead obtained by multiple distillation without ebullition[7].

Large samples of liquids free from particles could be produced from aerosols by separating contaminated from uncontaminated droplets. When the temperature of a monodisperse liquid aerosol is held slightly above the critical under-cooling point, all droplets containing particles are frozen. By ultra-sound of a wave-length comparable with the diameter of these droplets, the particle-free liquid droplets can then be sub-divided further. The resulting difference in particle size causes the contaminated particles to sediment before the uncontaminated ones.

Bulk samples of materials could similarly be purified from the last traces of a given homogeneous impurity. The properties of the latter determine the technique by which the aerosol particles are separated. In the case of impurities amenable to uninterrupted selective heating, it is advisable to utilize the very large negative temperature coefficient of the rate of crystallization of particle-free droplets. The noble gas, in which the monodisperse liquid aerosol is suspended, is cooled to the critical under-cooling temperature. Then only the contaminated droplets, which are subjected to induction or to high-frequency dielectric heating, remain liquid and can be dispersed ultrasonically. In other cases, for example, contaminated aerosol particles could be photo-ionized selectively by ultra-violet radiation, and electrostatically precipitated.

According to the dispersion principle, the fraction of particles free from impurities increases with the degree of dispersion. Volatile covalent compounds can be dispersed to discrete molecules within an involatile inert matrix. This makes it possible to purify small quantities of such compounds by fractional molecular distillation, in which these molecules are separately evaporated by selective infra-red radiation[11]. The high cost of this method limits it to laboratory use.

As a guest in this Institute, I thank Prof. W. Jost for his hospitality.

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Polyamide Layer Chromatography of Oestrogens

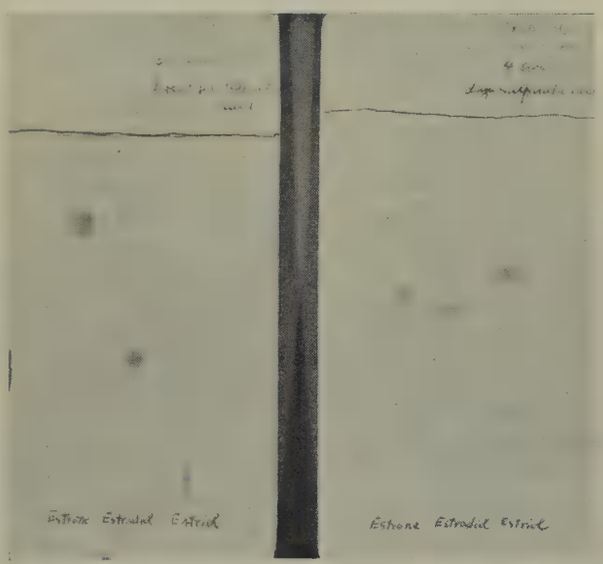
The oestrogens were investigated by Struck[1] and Lisboa *et al.*[2] by thin-layer chromatography using silica-gel. The solvent systems for the silica-gel thin-layer were well-established ones which have been used for quantitative purposes[3]. On the other hand, Woltz and Chattaraj[4] combined thin-layer and gas-chromatography to identify the minor oestrogenic substances in female urine.

Since oestrogens contain a phenolic group in their molecule, it is conceivable that polyamide can be used

Table 1

	Chloroform–butanol (95 : 5)	Ethanol–water (60 : 40)
Oestrone	0·82	0·35
Oestradiol (17-β)	0·55	0·32
Oestriol	0·21	0·48

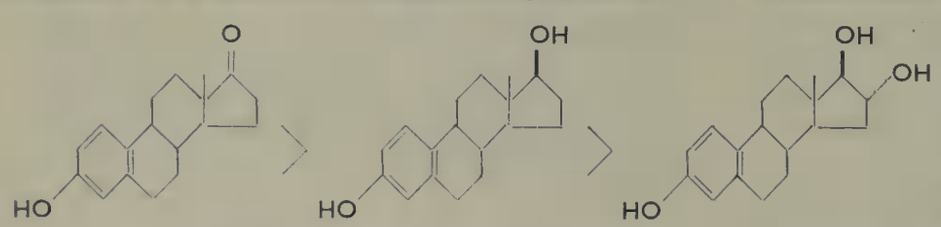
for their separation[5]. The polyamide layer, according to Wang[6], was applied to oestrone, oestradiol and oestriol, and both chloroform–*n*-butanol (95 : 5) and ethanol–water (60 : 40) systems showed excellent resolution (Table 1 and Fig. 1). This suggests that quantitative separation of oestrogens of complex biological fluid can be done by column chromatography with polyamide powder. It is also possible to investigate minor oestrogenic substances by means of two-dimensional polyamide layer chromatography.



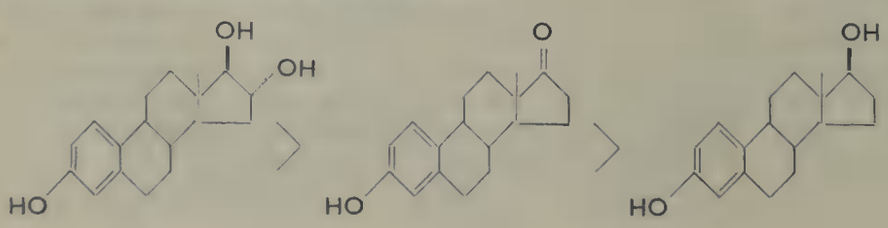
a *b*

Fig. 1. Polyamide layer according to Wang (ref. 6). *a*, Solvent : chloroform–*n*–butanol (95 : 5); time : 1·5 h (10 cm). *b*, Solvent : ethanol–water (60 : 40); time : 2·5 h (11 cm). Samples, 1, oestrone; 2, oestradiol (17-β); 3, oestriol. Detection : diazotized sulphanilic acid

It was stated by Carelli *et al.*[5] that hydrogen bonding of the phenolic group is the main force of polyamide sorption of phenols, and he has suggested a neighbour group 'screening effect'. If this is true, the oestrogens may show only a small difference on a chromatogram because they have a similar substitution pattern on the phenol ring. Differences occur, however, in the alcoholic hydroxyl groups, which are at distant sites on the 16- and 17-carbon atoms. Contrary to prediction, the chloroform–butanol system shows a marked difference in R_F values. It is best to assume that the alcoholic hydroxyl group in the oestrogens has some effect on their sorption to polyamide since an increase in hydroxyl groups also leads to an increase in the adhesion of substances to polyamide.



On the other hand, the fast-moving spot using an ethanol : water system is oestriol, and oestradiol is slower —oestrone is very close to oestradiol.



Although the presence of water affected the affinity of oestrogens for polyamide, it seems too early to suggest a polyamide sorption mechanism by the ethanol–water system as for the paper chromatographic-type partition process put forward by Copius-Peereboom[7]: in true partition by paper chromatography[8], oestrone is the faster and oestriol remains at the origin, while oestradiol is intermediate between the two. There is the same tendency with chloroform–*n*-butanol systems of polyamide-layer chromatography. Our experiments on oestrogens suggest that further elaboration of the sorption mechanism is necessary.

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Slow Inversion at the Nitrogen Atom in Certain Amides

It has long been considered that nuclear magnetic resonance (NMR) should be a suitable technique for the demonstration of slow inversion in trivalent nitrogen compounds. However, only in the case of cyclic imines, such as *N*-ethylethyleneimine, has it been possible to demonstrate slow inversion by NMR[1]. Recently, Saunders and Yamata[2] searched for evidence of slow inversion in dibenzyl methylamine, but found no evidence for non-equivalence of methylene protons in the amine itself. They were successful, however, in observing non-equivalence in the amine hydrochloride.

We wish to report evidence for slow inversion in the proton magnetic resonance spectrogram of *N*-benzyl-*N*-(orthomethyl) phenyl acetamide (I) and related compounds. The spectrogram of this compound is compared with those of *N*-benzyl acetanilide (II) and *N*-benzyl-*N*-(2,6-dimethyl) phenyl acetamide (III) in Fig. 1. The pertinent feature in the spectrogram of (I) is the multiplicity (four peaks) of the signal for the methylene protons. We have analysed these signals as an *AB* pattern with a chemical shift of 0·83 p.p.m. and a coupling constant of 14 c/s. The intensity ratio of the inner to outer lines is predicted to be 1·74 for such a pattern and was observed to be 1·67.

If a mechanism for a rapid exchange of identities of the methylene protons existed, the *AB* pattern would not be observable. Rapid inversion at the nitrogen atom is such a mechanism. From this it follows that inversion must be less rapid than a mean lifetime of something like 1–10 milliseconds.

The lack of observable non-equivalence in II and III indicates that a fairly high order of dissymmetry in the molecule is required. The requirement of a high degree of dissymmetry may be the reason that slow inversion has not been observed for acyclic trivalent nitrogen in the past. Evidently even the

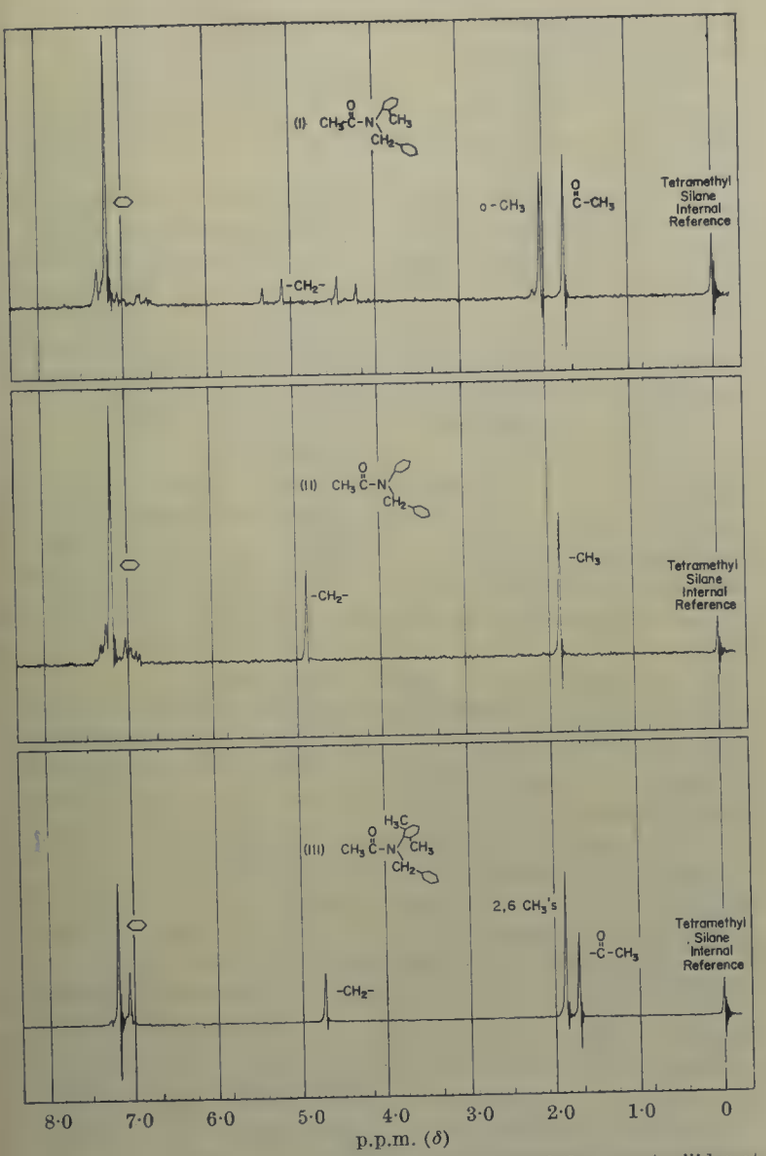

Fig. 1. Proton magnetic resonance spectrograms of three acetanilides at 60 mc/s

presence of three different substituents is not a guarantee of observable non-equivalence.

We have observed this type of non-equivalence in a number of related compounds and are continuing our study of this phenomenon.

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BIOCHEMISTRY

Proteins of Wheat Flour

A number of reports have recently been published concerning investigations of the 'zwickel' (wedge) and 'haft' (adhering) protein fractions of wheat flour and their influence on the separation of flour fractions of different protein content by air-classification procedures[1-4,11].

These fractions were originally described by Hess, who believed that flour or endosperm proteins in the native state existed in two forms, one as a network of fibrils on the surface of the starch granules and covered by a layer of lipid ('adhering' protein), and the other as discrete particles between the starch granules ('wedge' protein). These two fractions were readily separated by differential centrifugation in non-aqueous media and differed significantly from the equivalent fractions of the gluten isolate[8]. Hess concluded that during the conversion of native protein to gluten, mechanical treatments cause a structural chemical alteration of the protein molecules.

Jones *et al.*[9] have shown that flour fractions separated on the basis of particle size contain different amounts of protein. Up to about 17μ the particles include fragments of free protein with a relatively high protein-to-starch ratio. Particles of 17–35μ contain a large proportion of free starch granules and are consequently low in protein content. Above 35μ the particles consist of larger detached starch granules and pieces of unbroken endosperm cells and may have a similar or higher protein content than that of the parent flour[10]. These differences have provided the bases for the industrial air-classification of flours in the sub-sieve range ($< 40\mu$).

Results obtained by Stevens *et al.*[2], based on amino-acid analyses, indicate that proteins of the 'gliadin/glutenin' type are preferentially extracted in flour fractions of small particle size ($< 17\mu$), while the more soluble fractions appear to be associated with those fractions of larger particle size containing more starch. Protein analyses of Elton and Ewart, however, failed to indicate significant differences between these fractions[4].

A number of workers have recently examined the protein composition of air-classified fractions using some of the newer, refined techniques of protein fractionation in an attempt to confirm Hess's original classification[1,3,4,11]. In most cases the protein composition of these fractions differed little from that of the parent flour.

In this laboratory, the protein compositions of a number of air-classified flour fractions were examined by starch-gel electrophoresis following the methods described in previous reports[3,12]. Acetic acid extracts (2·0 g/6 ml., 0·01 N) were indeed found to vary little from those of the parent flour.

Samples of 'wedge' and 'adhering' protein obtained from the late Prof. Hess's laboratory when similarly examined however, showed significant differences in protein composition. 'Wedge' proteins corresponded mainly to endosperm components of low electrophoretic mobility (gliadins), while 'adhering' proteins corresponded to the slowest-moving endosperm fractions together with smaller levels of components of intermediate electrophoretic mobility (albumins). Densitometric scans of these starch-gel patterns are shown in Fig. 1.

Fig. 1. Starch-gel electrophoresis of 'wedge' (solid line) and 'adhering' (broken line) proteins of wheat endosperm. Buffer: aluminium lactate–lactic acid. Voltage gradient: 8–10 V/cm^{-1}. Time, 3 h

The obvious association of 'wedge' proteins with the generally accepted gliadin fractions and those fractions adhering to the starch granules with the albumins would seem to conform with recent findings. The fact that the 'adhering' protein appears to represent mainly a distinct group of components of lowest electrophoretic mobility would seem to confirm the original observations of Hess. Protein fractionation by air-classification would therefore seem to be much less complete when carried out on the present commercial scale.

A further interesting fact, which may be of some significance in this context, emerged from a comparison of the solubility characteristics of flour and isolated gluten proteins. Aqueous extracts of flour and gluten, isolated from an equivalent amount of the same flour, were shown to contain a rather different proportion of protein components (Fig. 2). The latter contained a greater proportion of the slow-moving fractions with the exception of those of lowest electrophoretic mobility. Again the fractions of

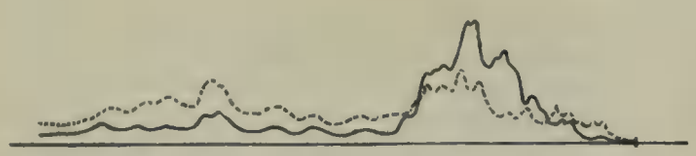

Fig. 2. Starch-gel electrophoresis of the water-soluble proteins extracted from flour (broken line) and the equivalent amount of isolated gluten (solid line)

lowest mobility were associated with the albumin components. This could not be explained merely in terms of starch-dilution effects. Similar examination of aqueous extracts of doughs aged for various periods gave protein patterns identical with those of flour. It would therefore seem that the removal of starch during gluten isolation involves not only the removal of the bulk of the water-soluble albumins but also the fractions of lowest electrophoretic mobility, that is, those fractions associated with the starch granules.

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Tumour-promoting Agents in Unburned Cigarette Tobacco

Unburned American cigarette tobacco is an excellent source of tumour-promoting agents[1]. The aqueous extract from as little as one-half cigarette per day is sufficient to produce skin tumours in mice painted previously with 125 μg of 7,12-dimethylbenz[α]anthracene (DMBA). The work recorded here gives further information about the properties of the active substance.

For most of the experiments, the paper was removed from commercial cigarettes and the tobacco was extracted with aqueous barium hydroxide which was afterwards neutralized with carbon dioxide and filtered. This method provides an extract containing 0·6–0·7 g of solids per ml.[1]. In one experiment, however, unprocessed commercial flue-cured tobacco was used in order to determine whether the tumour-promoting activity was due to the unburned tobacco itself, or to the several additives used in the preparation of American cigarettes. Flue-cured tobacco was less active than prepared cigarette tobacco as a source of promoting agents.

ICR Swiss female mice, 55–60 days old, were painted once with 125 μg of DMBA dissolved in 0·25 ml. of acetone. After a period of three weeks, the animals were painted five times weekly with 0·25 ml. of the various test solutions. The animals were examined weekly and the number and distribution of tumours noted. The experiments were terminated after 26 weeks of this second, tumour-promoting stimulus. The biological assay system has been described more fully in other publications[1,2].

Each test fraction was assayed at two concentrations, the second one-half the concentration of the first. In this way a semi-quantitative comparison of various fractions was possible.

An attempt was made to fractionate the aqueous extract into acidic, neutral, and basic materials by liquid–liquid extraction techniques. However, stable emulsions of organic and aqueous phases resulted, and it was decided instead to use ion exchange resins. The neutralized aqueous extract (133 c.c. from 267 g of tobacco) was made acidic (*p*H 1·5) with hydrochloric acid and the solution was then passed through a column (45 × 420 mm) containing a strong-acid cation exchange resin ('Rexyn *AG* 50(H)'). The column was afterwards washed with distilled water until the washings were colourless. The effluents from this column were then passed sequentially through a similar column containing a strong-base anion exchange resin ('Rexyn *RG*1 (OH)'). The second column was also washed with water. The effluents from the second column were condensed to provide the neutral fraction. The '*AG* 50' column was treated with 4 per cent barium hydroxide until Ba^{++} came through the column and the effluent was colourless. The combined effluent was neutralized with carbon dioxide, filtered and condensed to provide the bases plus amphoteric compounds. The '*RG*1-OH' column was washed with 1 N sulphuric acid until the effluent was colourless and the $SO_4^=$ came through the column; the combined effluent was neutralized with barium hydroxide, filtered, condensed and labelled '*A*' (acids). Half of each fraction was pooled to provide a reconstituted starting material that was identical to the starting material except for changes due to the experimental manipulations. The bioassays showed that much of the activity was lost (Table 1). It did not appear in any fraction or in the reconstituted crude material. The active material probably was either decomposed or not eluted from one of the columns.

A second experiment was conducted to determine whether the active material in the aqueous barium hydroxide extract would dissolve in acetone so that it might be more easily fractionated. Acetone was considered as a possible solvent because acetone–benzene extracts of cigarette tobacco have been found to be active tumour promoters[1]. When the aqueous extract was condensed to remove the water, acetone did not mix with the gummy product and incomplete extraction was apparent. There-

Table 1. Activity of 'Acidic', Basic, and Neutral Fractions

Fraction	Dose (tobacco equivalent per day)* (g)	No. of mice with tumours†	Maximum No. of tumours
Crude	0·5	6	12
Acids	0·5	1	1
Bases plus amphoteric compounds	0·5	0	0
Neutral	0·5	0	0
Reconstituted crude	0·5	1	1
Crude	0·25	1	1
Acids	0·25	0	0
Bases plus amphoteric compounds	0·25	0	0
Neutral	0·25	1	3
Reconstituted crude	0·25	0	1

* The amount of tobacco from which the various test solutions were obtained.
† Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

Table 2. Activity of the Acetone-soluble Fraction

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Crude	0·5	6	22
Acetone soluble	0·5	3	5
Acetone insoluble	0·5	0	0
Reconstituted	0·5	2	2
Crude	0·25	0	0
Acetone soluble	0·25	0	0
Acetone insoluble	0·25	0	0
Reconstituted	0·25	2	2
DMBA alone	0	1	1

* Forty mice per group; controls treated with the fractions alone did not develop tumours.

Table 3. Stability of Tumour-promoting Agents

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Fresh crude	0·5	3	3
*p*H 3	0·5	4	6
*p*H 7	0·5	5	15
*p*H 11	0·5	7	17
Fresh crude	0·25	0	0
*p*H 3	0·25	0	0
*p*H 7	0·25	0	0
*p*H 11	0·25	0	0

* Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

Table 4. Volatility of Tumour-promoting Agent

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Crude	0·5	3	3
Distillate	0·5	0	0
Residue	0·5	10	48
Reconstituted crude	0·5	13	41
Crude	0·25	0	0
Distillate	0·25	0	0
Residue	0·25	0	0
Reconstituted crude	0·25	1	1

* Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

fore, the dilute aqueous extract was treated with two volumes of acetone with continuous stirring, whereon a precipitate settled out. The solution was decanted and condensed to low volume and additional acetone was added, precipitating more material. This process was repeated three more times; each time additional precipitate formed. The final solution was condensed and redissolved in a 1 : 1 acetone–water mixture. The precipitates were pooled and dissolved in water. As before, a reconstituted starting material was prepared by using appropriate amounts of the acetone soluble and acetone insoluble fractions. The results of the bioassays (Table 2) indicated that at least a part of the active material appeared in the acetone soluble fraction. There was no demonstrable activity in the acetone insoluble fraction. A possible loss of activity during experimental manipulation was indicated by the decreased number of tumours produced by the reconstituted crude material and by the acetone soluble fraction.

To measure the stability of the tumour promoters, additional aqueous barium hydroxide extract was adjusted to *p*H 3, 7 or 11 using sulphuric acid or barium hydroxide. The solutions were then allowed to stand in subdued light at room temperature. After 5 days, they were neutralized with sulphuric acid or barium hydroxide, filtered and tested on mice. The bioassays showed no evidence that the tumour promoters were unstable (Table 3). In fact, there was an increase in activity during standing, especially at the highest *p*H level. This increase, however, was only moderate as indicated by low activity of the diluted samples.

The volatility of the active material was tested by distilling the neutralized aqueous extract at a pressure of 18 mm to a final temperature of 27° C. One-half of the distillate and one-half of the viscous residue were pooled to produce a reconstituted starting material. The remaining distillate and residue was diluted with water to the original volume from which it was derived. Under the conditions of the distillation, the tumour promoters were non-volatile (Table 4). The residues appeared to be more active than the starting crude product, but again, this increased activity was lost by 1 : 1 dilution. The distillate was inactive.

The results of these various experiments suggest to us the following tentative conclusions: (1) The tumour promoter of the alkaline aqueous extract and the acetone–benzene extract of cigarette tobacco are closely related, if not identical. (2) The active material is either very highly bound to one of the ion exchange resins, or it is insoluble at extreme *p*H so that it is not eluted by the concentrated reagents that we used. (3) The active material may be produced through the degradation of precursor materials in the extracts or in the leaf itself. There was a slight increase of activity when extracts were allowed to stand—particularly at elevated *p*H.

The biological assays of the active material indicated a definite threshold level. This is clear in every experiment. A 1 : 1 dilution caused almost complete loss of activity in every fraction. Thresholds are characteristic of tumour promotion and have been investigated extensively by Boutwell[3].

It was pointed out to us[4] that the behaviour of the active material is similar to the behaviour of tobacco pigments which have been found in several types of tobacco[5–8]. The pigments are soluble in alkaline aqueous solution but are precipitated at very low *p*H. They are, in part, soluble in alcohol. They are complex compounds containing the polyphenols rutin and/or chlorogenic acid bound to proteins or polypeptides. Conceivably, these pigment materials might be degraded on standing to liberate smaller polyphenolic compounds. Furthermore, relatively low molecular weight polyphenolic compounds have been found in flue-cured tobacco[6]. It is of interest that, other than croton oil constituents, the most active known tumour-promoting agent is the polyphenol, anthralin (1,8,9-anthratriol)[2]. We are at present testing tobacco pigments isolated by a method developed by Chortyk and Stedman[4] to determine whether these compounds or their hydrolytic products can account for the tumour-promoting activity exhibited by extracts of cigarette tobaccos.

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Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid

In bacteria, amino-acids are needed in small amounts for the synthesis of ribonucleic acid (RNA) to occur[1] and, if an amino-acid auxotroph is starved of its required amino-acid, both protein and RNA synthesis cease[2]. However, under certain conditions this 'stringency' is not maintained, in that protein synthesis can be sharply retarded while synthesis of RNA is less affected. In *Escherichia coli*, this differential synthesis of RNA can be brought about by incubation of cells with chloramphenicol[3], puromycin[4], or chlortetracycline[5], and mutants in which the control of RNA synthesis by amino-acids is 'relaxed' have also been described[6,7]. Relaxed mutants which require an amino-acid for growth continue to make RNA when starved of their amino-acid requirement; synthesis of RNA in a relaxed strain during inhibition of protein synthesis by 5-methyl tryptophan has also been demonstrated[8].

Differential synthesis of RNA has now been found to occur when cells of the stringent strain *Escherichia coli K*12 are inhibited by azetidine-2-carboxylic acid. This compound, while inhibiting growth, appears also to be incorporated into protein in place of proline[9]: in plants and rat liver the formation of an azetidine-2-carboxylic acid-transfer-RNA complex is apparently mediated by the

proline-activating enzyme[10]. Table 1 shows the results of adding azetidine-2-carboxylic acid (100 μg/ml.) to *Escherichia coli K*12 growing exponentially with a mean generation time of 70 min in a mineral-salts medium. There was an immediate slowing of protein synthesis, which, after 30 min, had been inhibited by 59 per cent; in this same period no inhibition of synthesis of RNA occurred. RNA was afterwards made at a reduced rate, but after incubation for 2 h in the presence of the analogue, the ratio of newly synthesized RNA to newly synthesized protein in the inhibited cultures was nearly twice that in the controls. Similar experiments were also carried out with the relaxed methionine-requiring mutant 58–161 of *Escherichia coli*. As shown in Table 1, addition of 100 μg/ml. of azetidine-2-carboxylic acid to cultures growing exponentially (mean generation time 75 min) produced an immediate strong inhibition of protein synthesis very similar to that observed in the stringent strain. However, RNA synthesis was initially stimulated appreciably and, although considerable inhibition developed at later times, this was less than in the stringent strain. Over a 2 h period, cells treated with the analogue synthesized about 2·5 times as much RNA per unit of newly made protein as did untreated cultures.

Table 1. Synthesis of RNA and Protein during Inhibition by Azetidine-2-carboxylic Acid

Organism	Time (min)	RNA (c.p.m./ml.) Control	RNA (c.p.m./ml.) Inhibited	Protein (c.p.m./ml.) Control	Protein (c.p.m./ml.) Inhibited	Inhibition (per cent) RNA	Inhibition (per cent) Protein
*E. coli K*12	30	140	140	60	25	0	59
	60	430	280	145	40	35	72
	120	1,175	455	360	75	61	79
E. coli 58–161	30	410	515	180	95	−26	47
	60	1,075	1,055	425	165	2	61
	120	2,465	1,815	975	305	26	69

Cells were grown overnight with aeration and at 30° C in a mineral-salts medium containing DL-methionine (50 μg/ml.) and a limiting amount (66 μg/ml.) of glucose. Excess glucose (7 mg/ml.) was added to re-start growth and, after 60 min, aliquots were transferred to flasks containing uracil and cytosine (final concentration 20 and 15 μg/ml., respectively) and aeration continued. RNA and protein synthesis were followed separately by the addition of [2-^{14}C]uracil (20 mμc./ml.) and [^{35}S]methionine (30 mμc./ml.) in the absence and presence of L-azetidine-2-carboxylic acid (100 μg/ml.). Measurements were made after the addition of the analogue and were of the radioactivity of cells precipitated with 5 per cent trichloroacetic acid at 0° C. Radioactivities were measured at infinite thinness with a gas-flow counter.

It has been suggested that the control of RNA synthesis by amino-acids may be mediated not by the amino-acids as such, but by their complexes with transfer-RNA. Species of transfer-RNA uncombined with amino-acids may cause repression of RNA synthesis; when molecules of transfer-RNA are joined to their specific amino-acids the repression is lifted and synthesis of RNA can continue[7,11]. On this hypothesis differential synthesis of RNA in the presence of azetidine-2-carboxylic acid may occur in the stringent strain *K*12 because the analogue can undergo activation and be incorporated slowly into protein. Although protein synthesis is inhibited, uncombined transfer-RNA molecules may not accumulate immediately and synthesis of RNA is not retarded. In the relaxed mutant 58–161, differential synthesis of RNA may be made greater by the genetic defect in the control of RNA which exists in this strain.

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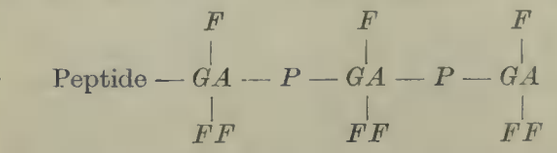
'Lipid *A*' Component from the Cell-walls of *Pseudomonas aeruginosa*

Considerable attention[1] has been paid to the lipopolysaccharide complexes which are found in the cell walls of Gram-negative bacteria. The extraction of whole cells or of isolated cell walls with aqueous phenol provides these lipopolysaccharides. The lipopolysaccharide component may be hydrolysed by dilute acid to give 'lipid *A*' and sugar. Nowotny[2] has suggested the following structure for the 'lipid *A*' derived from some strains of *Salmonella*.



F, fatty acid; *GA*, D-glucosamine; *P*, phosphoric acid.

Burton and Carter[3] have recently isolated the 'lipid *A*' fraction from whole cells of *Escherichia coli* and, while agreeing in the main with the structure postulated by Nowotny, they point out that their findings would suggest that the D-glucosamine units are linked glycosidically and not by means of phospho-diester linkages. The precise arrangement and the identity of some of the fatty acid constituents of 'lipid *A*' remain undetermined.

The results obtained in our laboratories during an investigation into the structure of the cell walls of *Pseudomonas aeruginosa* agree with those of Burton and Carter and would support the suggestion[4] that this type of structure is widely distributed in Gram-negative bacteria.

The cell walls of *Pseudomonas aeruginosa* were isolated by the method of Salton[5], and phenol extractions of the isolated cell walls were performed as described by Westphal[6], with the modification that the residue from the first extraction was again treated with phenol. The two phenol layers were combined and fraction *PhMP* was obtained by precipitation with methanol, while fraction *PhMS* remained in solution. The two aqueous layers were treated separately, giving rise to Aq I and Aq II as shown below.

Aq I	Aq II	Residue (*R*)	*PhMP*	*PhMS*
20–30%	11–13%	12–15%	20–28%	17–20%

Fraction *PhMS* contains 80 per cent lipid (cephalin and free fatty acids) and 20 per cent protein, while fraction *PhMP* is practically pure protein. The residue *R* is derived from the emulsion between the aqueous and phenolic layers and is a mixture consisting mainly of mucopeptide and protein. Detailed analyses of these fractions will be reported at some other time.

Although Aq I and Aq II showed some variation in quantity and elemental composition from extraction to extraction, all our information so far leads us to believe that they contain two lipopolysaccharide-mucopeptide complexes, and that Aq I and Aq II differ only in the relative proportions of these two major components.

The lipid fraction was obtained by mild hydrolysis of Aq I with boiling 1 N aqueous sulphuric acid for 30 min followed by extraction from the hydrolysate with chloroform, while the remaining aqueous layer contained D-glucose, L-rhamnose, D-glucosamine, and D-galactosamine. After removal of the acid from this aqueous layer and freeze drying, the infra-red spectrum of the residue revealed no indication of lipid material. Fractionation of the lipid on silicic acid gave two main fractions. The first fraction, presumably formed by partial degradation of 'lipid *A*', contained ester-bound fatty acid and free fatty acid. Thin-layer chromatography showed that the range of fatty acids was practically identical with that obtained by vigorous hydrolysis of Aq I with 6 N aqueous acid. This range included a number of acids, both saturated and unsaturated, up to about C_{16}, and also two unknown acids with high R_F values.

The second fraction was the whole 'lipid *A*', and the infra-red spectrum was almost identical with that obtained

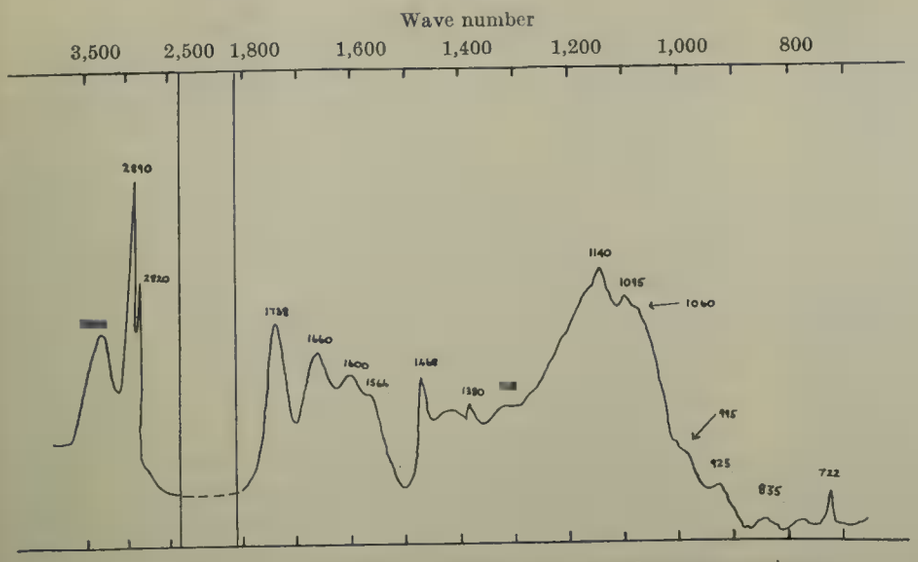


Fig. 1. The infra-red spectrum of 'lipid *A*' from *Pseudomonas aeruginosa*

by Burton and Carter for the 'lipid *A*' obtained by hydrolysis of the lipopolysaccharide from *Escherichia coli*. Examination of the infra-red spectrum of our 'lipid *A*' (Fig. 1), particularly in the region 750–1,000 cm^{-1}, shows the presence of moderate absorptions which Barker *et al.*[7] associate with glycosidic linkages. Our findings thus agree closely with those of Burton and Carter and would support their suggestion that the D-glucosamine units are linked glycosidically.

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Isozymes of Arylamidase (Aminopeptidase): Source of Blood Aminoacyl Naphthylamidase in the Rat

The qualitative approach provided by histochemical techniques makes it possible to localize enzyme activity in tissues. This, combined with the use of quantitative and other analytical methods such as electrophoresis, may help in tracing the origin and fate of an enzyme in the blood serum. This rationale has been applied in this study to certain proteolytic enzymes to which the names of aminopeptidase, arylamidase and aminoacyl naphthylamidase have been applied[1–3]. In this report the three names are regarded as being synonymous.

In the rat, blood levels of aminopeptidase vary within a narrow range in normal conditions[4]. There is electrophoretic evidence that renal aminopeptidase, localized in cells of the proximal tubules, is the source of the urinary isozymes which are distinct from the serum aminopeptidase isozyme[5]. Certain other cells of the rat, such as columnar cells of the small intestine, hepatic parenchymal cells, prostatic epithelium, parathyroid cells, fibroblasts, macrophages, mast cells and heterophiles, contain histochemically demonstrable aminopeptidase. In order to establish a correlation between serum and tissue arylamidases in the rat, blood serum and supernatants of homogenates of liver, prostate, small intestine mucosa, and skin were subjected to electrophoresis in a starch-gel medium.

Prostate was homogenized at a concentration of 400 mg/ml. and liver at a concentration of 600 mg/ml. A segment of jejunum was resected. The mucosa was rinsed out gently with saline and the intestine was then cut open lengthwise. The mucosa was scraped off with a razor blade. Fragments of mucosa were homogenized at a concentration of 400 mg/ml. Tissues were disintegrated for 10 min in a Potter–Elvehjem homogenizer. Homogenates were spun at 4,000 r.p.m. in a cold centrifuge at 4° C for 45 min. Supernatants were used as enzyme sources.

An area of skin was thoroughly shaved, and the epidermis scraped off. This area, resected without including muscle or subcutaneous tissue, was minced with a razor blade. 600 mg of skin were weighed out and transferred into a homogenizing tube to which 1 ml. of distilled water was added. The sample was homogenized for 10 min while the tube was kept in dry ice. 0·5 ml. of distilled water was added and, after homogenizing for 1–2 min, the supernatant was poured into another tube and the unhomogenized plug was discarded. The supernatant was spun in a cold centrifuge at 4° C for 45 min at 4,000 r.p.m. The clear supernatant was used.

Serum and supernatant of homogenates of these organs were subjected to zone electrophoresis in a starch-gel medium[6]. A 'Heathkit' regulated power supply was used. 6·5 V/cm was kept during the time of electrophoresis, which was 6 h, at room temperature. At the end of this period, gels were sliced and incubated for the demonstration of aminopeptidase as described in a previous publication[5]. 10 experiments were performed for each enzyme source. A diagram representing the average rate of migration of proteins with aminopeptidase activity is reproduced in Fig. 1.

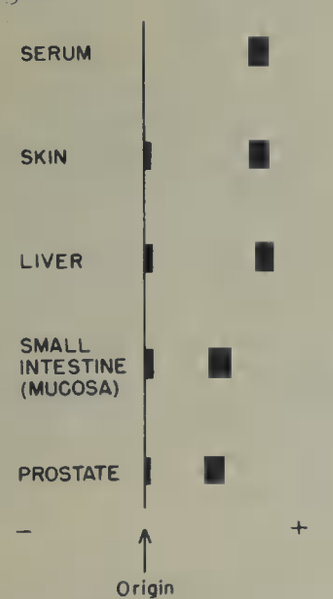

Fig. 1. Starch-gel electrophoresis of rat blood serum and tissues

Two isozymes were present in each of the organs that were studied; an isozyme remaining at the origin, and a migrating isozyme. The moving isozyme showed different but fairly similar rates of migration for each organ. The serum isozyme is most closely duplicated by the skin isozyme, suggesting that this organ might be the source of blood arylamidase.

Dermal fibroblasts showed characteristic reactivity for arylamidase[4] (Fig 2). These cells extended all through the dermis. They were of laminar appearance with pro-

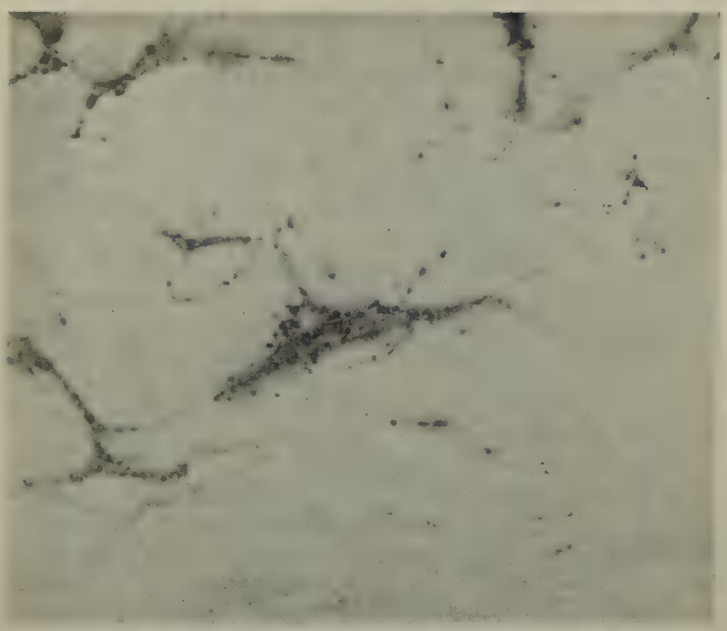

Fig. 2. Rat dermis. Aminopeptidase (L-alanyl-4-methoxy-2 naphthylamide HCl). Fibroblasts with processes intensely stained. There is an active vascular crevice in the upper left corner of this cell. (×1,250)

cesses extending along collagen bundles and abutting on small vessels in which endothelial cells were also active for aminopeptidase. This suggests that aminopeptidase of fibroblasts may be the main or only source of serum aminopeptidase.

It has been suggested on the bases of the study of similar enzymes in man that liver aminopeptidase might be the source of the serum enzyme[7]. It would seem probable that liver aminopeptidase, which by histochemical technique is localized in the peribiliary zone in dense bodies and in bile canaliculi, would be represented by the isozyme remaining at the origin. The moving hepatic isozyme resembled closely serum isozyme. Different results have been obtained for bile of man: one peak coinciding with the serum isozyme[8] or 2 peaks with incomplete resolution, one at origin and the other migrating like the serum isozyme[9]. Bile duct obstruction in man leads to a new aminopeptidase band at origin in serum[8,9]. This would suggest that the latter is a membrane-bound form which is also found in bile and would correspond to the bile canaliculi and dense body arylamidases.

It is concluded that, in the rat, the serum isozyme of aminopeptidase originates in the mobile isozyme of skin. Since fibroblasts are the main source of skin aminopeptidase, it is suggested that the soluble fraction of fibroblasts aminopeptidase is the main, if not the only, source of the serum isozyme, and that electrophoretically mobile liver isozyme represents cleared enzyme from serum appearing in bile. Experiments are being made to test this hypothesis.

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Evidence for the Release of Individual Amino-acids from the Resting Human Forearm

The study of differences in concentration of substances in arterial and venous blood is well-documented, and offers a method for investigating peripheral metabolism.

In order to study this aspect of amino-acid metabolism in man, cannulation of the brachial artery and brachial vein of the same limb[1] was carried out in six subjects, four of whom were normal, one convalescing from a myocardial infarction and one suffering from benign essential hypertension. All the experiments were carried out at 10 a.m., after an overnight fast. Two simultaneous arterial and venous blood samples were withdrawn from each individual; there was an interval of 30 min between the pairs of specimens. Immediately before and again after a blood sample had been taken, the forearm blood-flow was measured by plethysmography with a mercury-in-rubber strain-gauge[2] and was shown to be constant. During the blood-flow measurements and while the samples were being taken, an occluding cuff around the wrist was inflated to a pressure that was 100 mm mercury above the systolic blood pressure. Soon after the blood had been withdrawn, it was centrifuged in the cold, the plasma protein precipitated with 1 per cent picric acid[3] and stored at −15° C until analysed by automatic ion-exchange chromatography[4]. The means of the two arterial and the means of the two venous amino-acid measurements from each person were used for calculating the mean differences found in the group.

Table 1 shows the mean difference in venous and arterial amino-acid concentrations, expressed as a percentage of the arterial concentration, and also the differences themselves, in μmoles/100 ml. plasma, in the group of six subjects. The significance of the differences, calculated by testing against the null hypothesis using both tails of the '*t*' distribution[5], is also shown. During the time taken for the experiment there was no evidence of a downward drift in either arterial or venous concentrations. The venous levels were, apart from alanine, within the range delineated in a large range of normal men and women[6]. In the present series of experiments the range of alanine concentrations was 21·2–29·3 and the mean was 26·3 μmoles/100 ml.

Table 1. Differences in Concentrations of Plasma Amino-acids (Venous–Arterial) in the Resting Human Forearm

Amino-acid	Diff. (per cent)	*P*	Diff. (μmoles/100 ml.)	*P*
Aspartic acid	−6	0·6	−0·24	0·3
Threonine	13	0·025	2·03	0·02
Serine*, asparagine and glutamine	7	0·05	0·89	0·025
Glutamic acid	0·4	0·6	0·34	0·8
Glycine	17	0·01	3·37	0·005
Alanine	27	< 0·001	5·42	< 0·001
α-Aminobutyric acid	1	0·8	0·01	0·8
Valine	5	0·05 < *P* < 0·1	0·95	0·05 < *P* < 0·1
Cystine	2	0·4	0·02	0·7
Methionine	16	0·01	0·30	0·01
Isoleucine	7	0·05 < *P* < 0·1	0·39	0·05
Leucine	11	0·01	1·14	0·005
Tyrosine	12	0·2	0·40	0·2
Phenylalanine	16	0·005	0·67	0·005
Ornithine	2	0·6	0·11	0·6
Lysine	13	0·025	2·02	0·02
Histidine	11	0·005	0·82	0·005
Arginine	16	0·025	1·28	0·02

* Estimated as serine.

The results indicate that, under the experimental conditions used, there is for some amino-acids, including many that are considered to be essential, a significantly higher concentration in venous than in arterial blood plasma; thus at rest and while fasting there is a release of individual amino-acids from the human forearm.

Furthermore, since there is a difference between arterial and venous plasma in these experiments, it may be important to measure arterial concentrations in investigations of movements of amino-acids between intra- and extra-vascular sites.

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PHYSIOLOGY

Quantum Energy Specificity of Simple Photoreceptor Units

The compound lateral eye of *Limulus polyphemus* has been useful in basic visual function research[1,2]. *Limulus* ommatidia share similarities in photopigment[3], scotopic luminosity function[4], lateral neural inhibition[2], and other functions with the scotopic mammalian retina. Light excitability of *Limulus* functional visual receptor units may be modified reversibly by doses of X-rays smaller than required for visual cell pathology[5]. X-ray excitation of *Limulus* ommatidia[6] and vertebrate retinae[7] has been reported.

Electrophysiological examinations of more than 100 single and multiple ommatidium preparations have demonstrated inter- and intra-preparation X-ray sensitivity variations in contrast to relatively stable visible light-excitability. Records of single optic axon spike responses to whole lateral eye X-irradiation suggest that the minimal threshold may be low relative to common human exposures. Approximately 0·025 r. is delivered in a routine dental radiographic exposure. Fig. 1 describes the stimulus-response relationship for an X-ray sensitive, dark adapted ommatidium. X-ray parameters were 50 kVp., half-value layer 0·2 mm aluminium. Abscissa, absorbed dose was measured as the incident minus the transmitted dose accumulated during the two-second stimulus. Lateral eye preparation and maintenance have been described elsewhere[1,5,8].

It was common to find axons of individual ommatidia which were refractory to X-irradiation doses six log units greater than Fig. 1 threshold and three log unit X-ray intensity variations. The same neural units discharged vigorously during moderate visible stimuli (~10 lux). With less frequency, whole lateral eyes have been tested the optic nerves of which responded with a normal spike output to light flashes but remained largely quiescent during X-irradiation. In these cases it was not possible to record from all optic neurones.

The optic axon potentials in Fig. 2 were recorded simultaneously from X-ray sensitive and insensitive receptor units. Light stimulation was provided by an *R*1131*C* (Sylvania) glow modulator tube driven by a variable square wave. The stimulus spot diameter was reduced to 75μ at the cornea. Programmed stimulation began with a 1·7-sec light flash, followed by a 100-sec interval, and then a 4-sec X-ray exposure. A visible test flash was superimposed on the X-ray stimulus. Four records were taken at

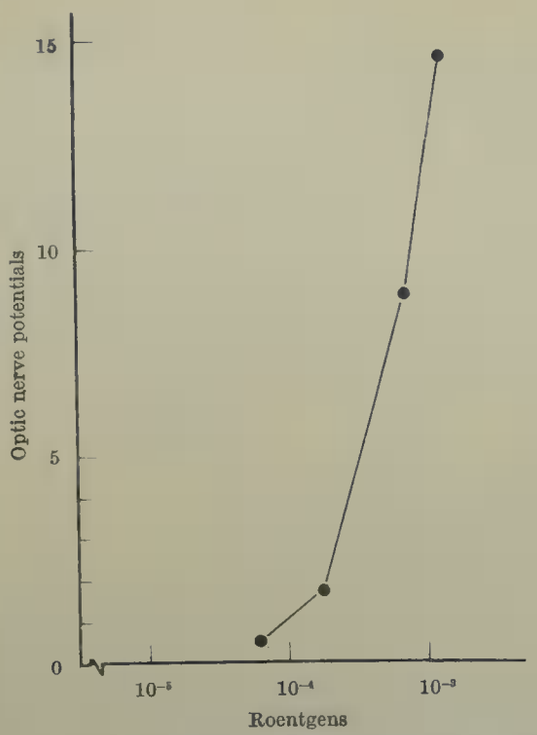

Fig. 1. Stimulus-response relationship for X-ray initiated optic nerve unit activity. Intensity of the two-second X-ray flash was varied by target-eye distance. Datum points are the means of four measures. The potentials indicated single unit activity

X-ray intensities intermediate to *A* and *B* of Fig. 2. Before *A* was recorded both receptors were located by the spot of light and it was determined that both axons responded similarly to the standard visible stimulus. Discharges indicative of two axons are present and may be identified by the degree of positive overshoot (downward deflexion). The unit which produced the greater overshoot emitted consistent response patterns to the standard visible test flash although the second light stimulus in each record was superimposed on and preceded by X-irradiation. Its counterpart responded during X-irradiation and more vigorously as the X-ray stimulus intensity increased.

Reports of retinograms and other retinal potentials may be interpreted to support X-ray excitation of the visual cell by a rhodopsin reaction[7]. Rhodopsin has been established as the photopigment of the *Limulus* ommatidium[3], but its action spectrum has not been extended into the X-ray band. Dartnall has suggested that 'inadequate' quanta are not absorbed by the rhodopsin chromophore[9]. The X-ray quanta probably exceed the energy required for photochemical activation which becomes a matter of absorption probability in the chromophore.

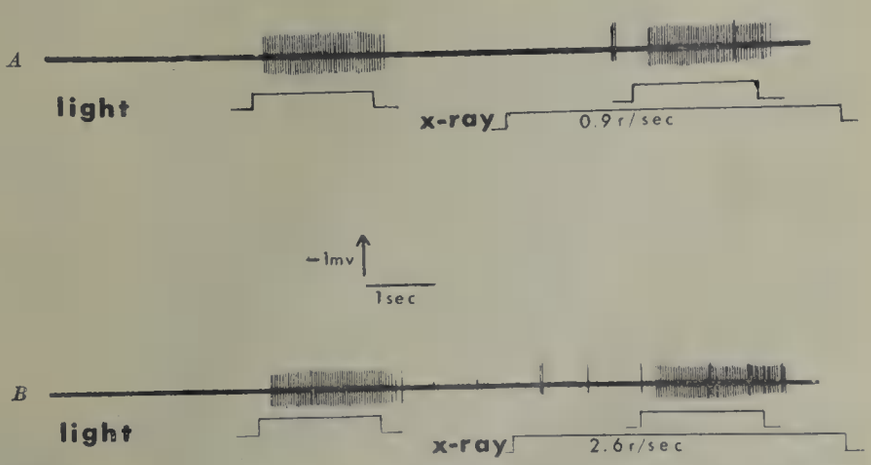

Fig. 2. Optic nerve axon potentials recorded simultaneously from two functional unit axons. Light stimulation was confined to one ommatidium. The whole eye received X-rays. In *A* the unit with greater positive overshoot responded to light but was not activated by X-rays which excited the unit axon identified by the smaller positive overshoot. Events in *B* were similar where X-ray intensity was increased. Some potentials were darkened for reproduction

However, quanta of higher energy than those of the visible band may be absorbed in photostable portions of the rhodopsin molecule[10].

It is difficult to explain consistent X-ray stimulus specificity by rhodopsin bleaching. Direct high-energy quantum interaction with the neural apparatus would account for numerous unexplained reports of X-ray avoidance behaviour in animals[11] and sensory phenomena in human beings[12].

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Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction

The depolarization of motor nerve terminals by the application of a cathodic current increases the frequency of spontaneous miniature potentials (m.e.p.p.s), but under these conditions the number of such quantal units of transmitter released by a nerve impulse is decreased in proportion to the intensity of the presynaptic depolarization[1–3]. Although the depolarization of the nerve terminal by elevating the potassium concentration also causes an increase in the frequency of m.e.p.p.s, the potentiation in this case continues to develop even after depolarization has reached a maximum[3,4]. Although equivalent depolarization can be achieved both electrically and chemically, the effects of these stimuli are not entirely parallel, as the quantum content of the end-plate potential is decreased in the first case but is potentiated in the second[1,2,5].

Since it is evident that presynaptic depolarization alone does not account for the facilitatory effect of potassium, the question naturally arises as to whether this cation may affect some other specific process in the mechanism of transmitter release. The work recorded here was carried out in order to determine whether the action of potassium could be accounted for by an increase in the probability of stimulus-evoked transmitter release.

Investigations of the effects of potassium on neuromuscular transmission were performed *in vitro* on the rat diaphragm-phrenic nerve preparation as described by Bülbring[6]. Rats of the Wistar strain, unselected as to sex, weighing 200–300 g, were used in these experiments. The left hemidiaphragm with its phrenic nerve intact was removed under acute ether narcosis and immediately immersed in a physiological solution[7], which was gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide by means of a lift pump system similar to that described by Szekeres and Vaughan Williams[8]. The temperature was maintained at 33° ± 0·1° C throughout the experiments.

Resting membrane potentials and transients were recorded by conventional techniques[9,10]. E.p.p.s and m.e.p.p.s were displayed at high amplification and photographed from one beam of an oscilloscope. To record the depolarization caused by a given concentration of potassium and to be able to reject fibres damaged during electrode placement, the membrane potentials of all fibres were continuously monitored on a second beam, which received signals through a direct-coupled amplifier. End-plate potentials were evoked by stimulating the phrenic nerve with repeated supramaximal stimuli delivered at frequencies of 25 or 50 c/s for 1–2 sec, and recordings were made when neuromuscular transmission was partially blocked either by the combined action of 10 mM magnesium and 1·5 mM calcium, or by curare, present in concentrations of 2–5 × 10^{-7} g/ml. The addition of magnesium chloride, calcium chloride and potassium chloride was osmotically compensated for by omitting appropriate amounts of sodium chloride from the medium[11].

The number of transmitter quanta released by a nerve impulse was estimated in two ways according to the conditions of the experiment. When neuromuscular transmission was blocked with high concentrations of magnesium, the quantum content m of an e.p.p. was determined from the ratio of the mean e.p.p. to the mean m.e.p.p. amplitude. In curarized preparations, the estimate was made from the ratio of the variance to the mean amplitude obtained in trains of impulses[12]. The concentrations of magnesium and curare used in the present work so reduced the postsynaptic depolarization by the e.p.p. that correction for non-linear summation was not necessary. The errors introduced by post-synaptic depolarization and the loss of small m.e.p.p.s into base-line 'noise' were neglected, as both factors were small.

When neuromuscular transmission was partly blocked with magnesium, an increase of the external potassium concentration from 5 to 15 mM produced a rise in the spontaneous quantal discharge frequency (m.e.p.p.s) from 5·8 to 20·8 sec^{-1}, and an increase in the mean number of quanta per e.p.p. from 4·2 to 7·2 (Table 1). To determine whether the facilitation of stimulus-linked release resulted from an increase in the size of the available pool of preformed transmitter quanta or whether it was due to a greater probability of release by a given impulse, additional experiments were performed with partially curarized preparations. It could be expected that the rate of initial transmitter depletion during a tetanus would increase if only the probability of release were affected, but would decline if the potassium excess had mobilized a greater number of pre-formed quanta or had speeded the reloading of vacated sites. If, on the other hand, the pool of transmitter were simply increased while the nerve impulse

Table 1. Effect of Potassium on Spontaneous and Stimulated Transmitter Release at 33° C

	Normal solution 5 mM K^+	High potassium solution 15 mM K^+
m.e.p.p. freq. (sec^{-1})	5·79 ± 1·14	20·81 ± 2·64
No. fibres	(14)	(16)
Mean RP (mV)	79·0	54·3
Mean m*	4·15 ± 0·75	7·19 ± 0·47
No. fibres	(15)	(26)
Mean RP (mV)	79·1	55·6

* Neuromuscular conduction partially blocked with 10 mM Mg^{++}, 1·5 mM Ca^{++}; m calculated as mean e.p.p. amplitude/mean m.e.p.p. amplitude during repetitive stimulation at 25 c.p.s.

Table 2. Effect of Potassium on Sustained Transmitter Release at 33° C

	Normal solution 5 mM K^+	High potassium solution 15 mM K^+
Mean RP	73·4	52·2
Mean m	70·0 ± 10·8*	132·8 ± 14·9
No. fibres	(14)	(20)

* Neuromuscular conduction partially blocked by d-tubocurarine 2–5 × 10^{-7} g/ml.

m Calculated from the ratio of the variance to the mean e.p.p. amplitude during repetitive stimulation at 50 c./s.

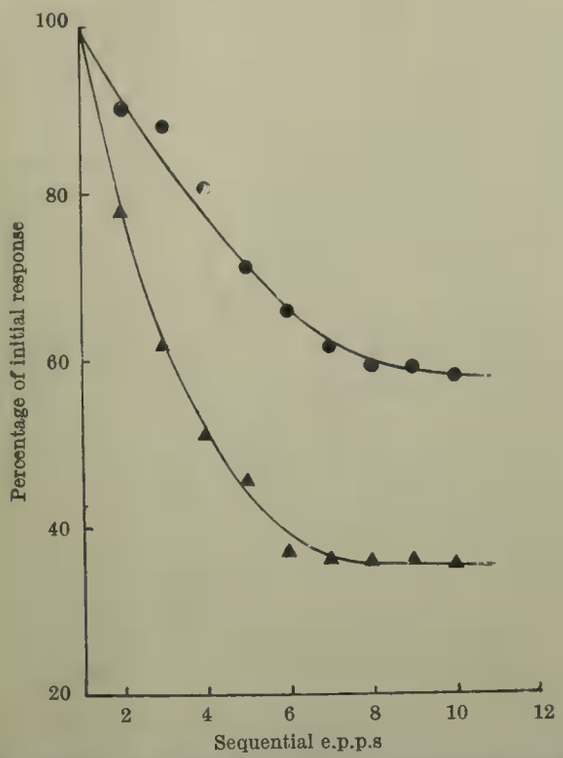

Fig. 1. The effect of potassium ions on the rate of initial transmitter depletion at 33° C. ●, Control solution containing 5 mM K^+; ▲, high potassium solution containing 15 mM K^+. Each point represents the average of several fibres plotted as per cent of mean initial end-plate potential amplitude. Stimulation rate 50 c/s

continued to release a constant fraction of transmitter, the depletion rate would remain stable and the absolute number of quanta released by successive impulses would rise.

It can be seen in Fig. 1 that the rate of depletion of transmitter during a tetanus was significantly increased when the potassium concentration was raised from 5 to 15 mM. The absolute number of quanta escaping per initial nerve impulse was nearly doubled by this treatment, the value of m rising from 117·3 ± 18·7 to 249·0 ± 20·2, and the level of sustained transmitter output, estimated from trains of impulses after an initial rundown, rose from a value of 70·0 to 132·8 (Table 2).

These results indicate that the potassium has at least two direct effects on neuromuscular transmission. It clearly increases the probability of release of transmitter by a nerve impulse and it also raises the number of quanta of transmitter in the readily releasable store.

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Prolonged Administration of Atropine or Histamine in a Silicone Rubber Implant

In the use of drugs, it is often desirable to maintain a constant blood and tissue concentration of chemical for prolonged periods. Several attempts to avoid the 'saw-tooth' oscillations of drug-levels that accompany periodic dosing have been reported. Daily injections of histamine in a beeswax repository have been used to develop gastro-duodenal lesions in dogs[1]. Thompson *et al.*[2] found that a single intramuscular injection of highly insoluble pamoate salt (*C*1–501, *cyclo*guanil pamoate) in mice and monkeys was successful as a repository drug against malaria. A constant level of medication, using silicone rubber as a carrier, was attempted by Folkman and Long[3,4] for cardiac pacemaker drugs and by Powers[5] in treating experimental malaria and schistosomiasis. We chose to investigate further the practicability of using silicone rubber to effect the slow release of drugs. Atropine and histamine were chosen for the study because they gave diverse but measurable responses, mydriasis in rats and gastro-intestinal lesions in dogs.

Atropine base was placed in tubes of silicone rubber ('Medical Silastic 372', 0·140 in. intern. diam. × 0·236 in. extern. diam.) (kindly supplied by Dow Corning Corp., Midland, Michigan), 3–6 cm in length to accommodate varying amounts of drug. Ends of the tubes were sealed with silicone medical adhesive. These tubes were washed in water, sterilized in benzethonium chloride, 1 : 750 ('Phemerol', Parke, Davies and Co., Detroit, Michigan) for 24 h, and placed in sterile distilled water for 24 h.

Thirteen treatments consisted of: subcutaneous tube implants of atropine base (powder) at 400, 200, 100, 50, 25, 12·5 and 6·25 mg/rat; implants of empty tubes plus subcutaneous injections of atropine base suspended in aqueous 1 per cent methyl cellulose in doses of 400, 100, 25 and 6·25 mg/rat; 2 carrier control groups: (*a*) empty tube implants; (*b*) empty tube implants plus subcutaneous injection of 1 per cent methyl cellulose. Tubes were placed in a subcutaneous channel between the scapulae, and the skin incision was closed with wound clips. The thirteen treatments were assigned to 78 Sprague–Dawley male rats according to a randomized block design with a total of six blocks. The animals were housed, one block per cage, in a room with constant temperature and humidity.

Following implant, daily observations of pupil dilatation were made with an ophthalmoscope without knowledge of individual treatment. The degree of mydriasis was recorded as one of five possibilities. The normal pupil received a rating of 0, and 25, 50, 75 and 100 per cent dilatations were rated +1 to +4 respectively. The values recorded in Fig. 1 represent the number of days each treatment group had a mean response equal to or greater than +1 mydriasis.

Atropine, enclosed in 'Silastic', produced a dose-dependent mydriasis. Duration of mydriasis was longer with the 'Silastic' tube implant than with the atropine injection. For example, at the 100 mg/kg dose-level, the 'Silastic' tube group had mydriasis for twenty-six days while the injected group had only six days of mydriasis (Fig. 1). The control groups had mydriasis for one day which was probably due to their eating animals that had had received a lethal dose of atropine.

Mortality was dependent on mode of administration. There were no deaths among the rats receiving atropine in 'Silastic' tubes. When atropine was injected subcutaneously, 400 mg was fatal to 6/6 rats and 100 mg was fatal to 3/6 rats. Animals which received injections had severe necrosis at the injection site.

Attempts were made to induce gastrointestinal lesions with histamine implants in dogs of mixed sex and breed weighing 8–10 kg. Histamine (Calbiochem, Los Angeles) was placed in silicone rubber tubes or moulded with '*RTV* 5392' (Dow-Corning, Midland, Michigan). To

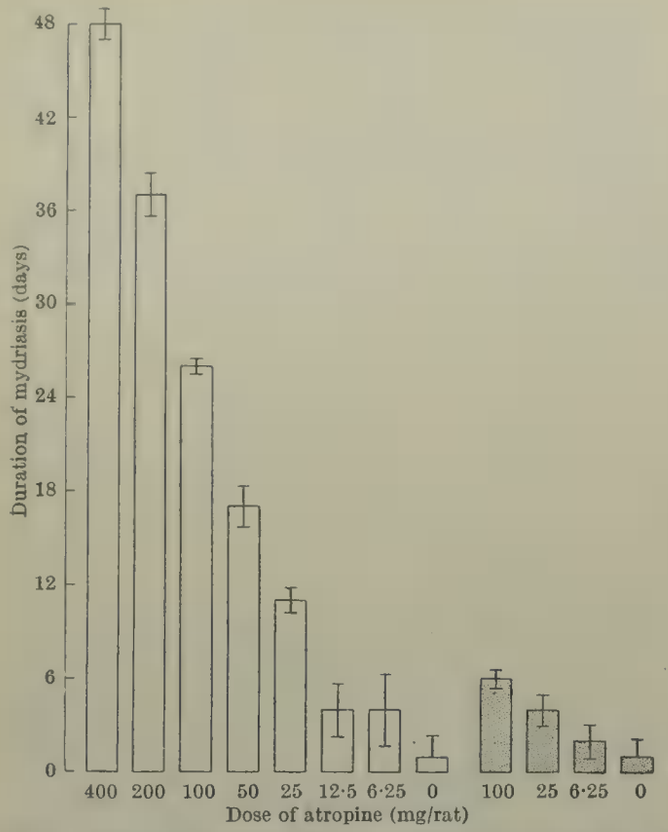

Fig. 1. Duration of mydriatic response in days ± *S.E.* to graded doses of injected (stippled bars) and silicone encapsulated (plain bars) atropine in rats

form the mould, 250 mg histamine base was triturated with 5 c.c. of the liquid silicone rubber, drawn into a plastic syringe and allowed to vulcanize. The plastic syringe was broken to remove the mould. All implants were sterilized as already described here. The tubes, a mould, or a tube plus a mould were implanted subcutaneously between the scapulae. At autopsy the stomach and duodenal areas were examined macroscopically for lesions. The results are shown in Table 1.

Table 1. Ability of Various Forms of Histamine Implants to induce Lesions on the Gastro-duodenal Area of Dogs

No. of dogs	Method of administration	Amount of histamine (g) per implant	No. of implants	No. of days between first implant and autopsy	Lesions
6	Tube (T)	1–2	1	46–68	Neg.
2	T	2†	1	63	Neg.
2	T	0·5	5*	42	Perforated duodenal
2	Mould (M)	0·5	1	42	Neg.
1	T+M	0·5-T+0·25-M	5*	42	Neg.
2	T+M	0·5-T+0·25-M	4–5*	30–42	Antral and duodenal

* The implants were placed subcutaneously at intervals of one week and autopsy performed one week after the final implant.
† Dose divided among 4 tubes, and implanted subcutaneously in a single operation.

Six dogs received implants of a single tube containing 1 or 2 g of histamine; two dogs received 2 g of histamine divided among four tubes. None of these animals showed gross changes in the appearance of the gastrointestinal mucosa when autopsied 46–68 days after implant (Table 1). Weekly implants of single tubes containing 500 mg histamine into two dogs caused perforated duodenal lesions within 42 days.

The implant of a histamine mould gave negative results. Weekly implants of a tube plus a mould produced lesions in two of three dogs. One had multiple antral and duodenal erosions, the other developed one large and one perforated duodenal lesion (Table 1).

Histamine repositories were evaluated for their effects on gastric secretions in dogs prepared with denervated fundic pouches. Single tubes of 500 mg of histamine base were subcutaneously implanted into three dogs. Secretions and acid outputs were monitored periodically during the following eight days and compared with pre-implant rates. In a second test, a mould of 250 mg histamine base in '*RTV* 5392' was implanted into each of two dogs. Secretions were monitored for 12 days. Neither the tubes nor the moulds had any prolonged marked effect on secretion. The moulds caused a slight increase in secretion on the first day only, probably due to surface-trapped histamine.

Each of four dogs was implanted subcutaneously with four 'Silastic' tubes containing 500 mg histamine (2 g total). One dog showed no change in secretion for 21 days. The second dog had a slight increase in secretion from day 7 to day 37 which returned to basal level. The third dog had an increase in secretion by the 13th day. When killed on the 19th day, numerous large craters were found in the antrum. The gastric pouch and duodenum were normal in appearance. The fourth dog had an increase in gastric secretion by the 15th day. By the 21st day, the animal was losing weight, appeared dehydrated, and had several clonic convulsions. No lesions were found in the gastrointestinal tract.

Like other drugs[3,4], atropine encapsulated in a 'Silastic' jacket can exert a slow and prolonged biological response. The lack of effect after a single histamine 'Silastic' tube implant on lesion production or sustained gastric secretion invalidates this type of implant for inducing experimental lesions in dogs. The level of histamine needed to induce lesions or maintain gastric secretion may be critical and cannot be attained by a single implant. The weekly histamine implants did induce gastrointestinal lesions in 4 out of 5 dogs. This type of implant regimen was not tested for its effects on gastric secretion. Chemical determinations will be required to confirm drug-levels in tissues resulting from a repository implant.

A major advantage in using silicone rubber as carrier is that the 'encapsulated' drug can be easily removed from the animal which provides constant control over the duration of exposure to the drug. Waitz *et al.*[6] demonstrated a similar control with a dialysis bag placed in the peritoneal cavity of rats.

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Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation

The time interval between successive stimuli has been shown in a variety of situations to be crucial for the amplitude of response. For example, at certain intervals, the earlier stimulus may increase the amplitude of the response to the second stimulus. Such a phenomenon is known as facilitation, and has been demonstrated at the neural level, for example, by Lloyd[1,2].

In the experiment to be reported here, the time between two successive trains of electrically rewarding stimuli was varied to assess the effects of temporal spacing between them on a threshold of lever pressing.

The subjects were three male albino rats of the Sprague–Dawley strain, weighing about 250 g at the time of operation. Monopolar stainless steel electrodes, insulated except for 0·2 mm at the tip, were implanted. The co-ordinates aimed for were *A* 3·0, *L* 0·8, *H* 3·2 (Nos. 7 and 21) and *A* 2·5, *L* 1·0, *H* 3·2 (No. 38) according to DeGroot's atlas[3].

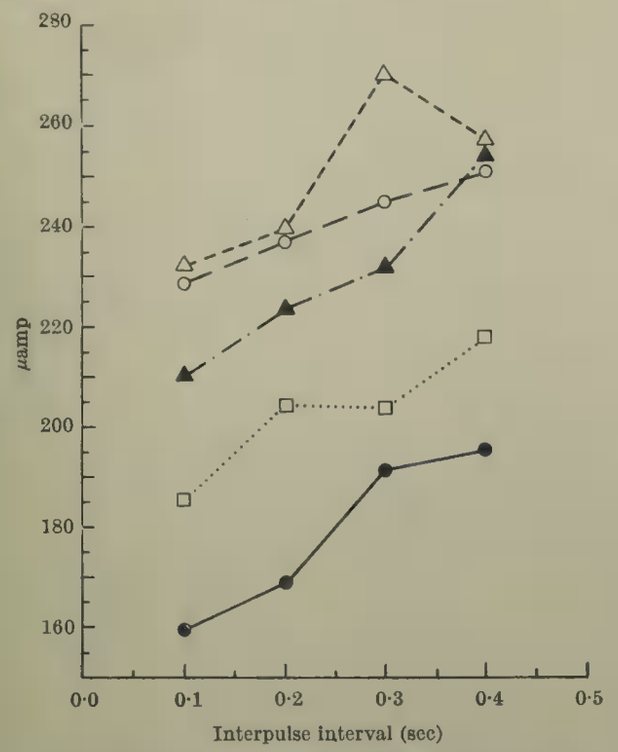

Fig. 1. The curves show how the threshold for the rewarding effect of intracranial stimulation increases as a function of separation of two 0·1-sec bursts of stimulation. The two uppermost curves for Nos. 38 and 7 show the result when the second train occurs 0·9 sec after the lever press. The other curves show the result when the first burst occurs immediately after a lever press is made. The two trains were only available once per second in both conditions. △ - - - △, No. 38; ○ – – ○, No. 38; ▲ – · – · – ▲, No. 7; □ · · · · · · □, No. 21; ■——■, No. 7

The rats were trained to press a lever in a Skinner box. A press delivered a 60-c/s large-amplitude stimulus lasting for 0·5 sec. The next stimulus was not available until 0·5 sec after the termination of the previous stimulus. The intensity of the 60-c/s stimulus was so adjusted that when the stimulus was disconnected after a session lasting one minute, the animal would continue pressing for well over a minute. As the method used has already been described[4,5], it will be only briefly recapitulated. Short training sessions were given at the end of each of which the current was disconnected. Such training continued until the rat left the bar within a few seconds after the current was switched off. When such training was complete, a session lasting one minute, with 60-c/s stimulus at the high intensity previously used, was followed by a test period of a further minute. During the test minute, the animal could only obtain, if it pressed, a certain intensity of two 0·1-sec trains of 60 c/s. (It is found that below a certain intensity the animal will act as if the current had been disconnected and above this level will tend to keep on pressing to obtain intracranial stimuli.) The criterion taken for the cessation of pressing (behaviour on the part of the animal as if no stimulation was obtained) was the execution of no responses at all for a continuous 30-sec period during the test minute. Reward threshold was then taken to be the step in intensity of the two 0·1-sec trains just above that at which the rat met the criterion of cessation of bar pressing. The stimuli which the rat could obtain during the test periods consisted of two 0·1-sec trains of 60 c/s separated by intervals of 0·1, 0·2, 0·3 and 0·4 sec. The maximum rate at which such train couples could be obtained was once per second.

The experiment was run under two main conditions. In one condition, the first train occurred as soon as the animal pressed the lever, at the beginning of the one-second interval which had to elapse before a further stimulus couple was obtainable. In the second condition (used to control for differential average delay of reinforcement with different intervals between the two 0·1-sec trains with Nos. 7 and 38) the last pulse was always given at the end of the second after the animal had pressed. In this way the average length of delay of reinforcement after a response was now longest for the 0·1-sec interval between trains, whereas in the first condition when the first train began at the beginning of the lever press, it was the shortest. A descending method of limits was used and the order of intervals (0·1–0·4 sec) was presented randomly from session to session.

The control of the timing of stimuli was achieved by using 'Digibit' logic panels. The 60-c/s stimulus was connected to the animal through a 'Variac' transformer and an isolation transformer. A 200-kohm resistor was placed in series with the electrodes. The stimulus to the animal was monitored on a 'Tektronix 502' oscilloscope.

The results are presented in Fig. 1. An analysis of variance was performed on the results of each individual animal in each of the two conditions separately. All the results indicate a linear trend (of current against interval between trains), the lowest level of significance being at the 0·01 level of confidence, no other effects being significant at this level.

It seems that the threshold for the rewarding effects of intracranial self-stimulation decreases the closer the temporal spacing of two identical stimulus trains. Though at present the relation of such a phenomenon to neural facilitation cannot be inferred with certainty, it seems most likely that the facilitation here observed occurs in the neural system subserving rewarding intracranial stimulation. The length of interval over which facilitation occurs is in excess of 0·4 sec. However, it is difficult to compare such a figure with others obtained under other conditions.

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IMMUNOLOGY

A Specific Agglutinin in the Snail *Otala* (*Helix*) *lactea*

Blood group specific agglutinins (lectins) were found some years ago in various plants[1–3]. Such substances have been found also in invertebrate animals[4], the most recent report being that by Johnson[5] of a weak anti-A_1 in the clam *Saxidomus giganteus*.

We have found that the expressed body fluids of the land snail, *Otala lactea*, contain a powerful and specific anti-A agglutinin (titre for A_1 erythrocytes 8,000, for A_2 2,000). This agglutinin, even undiluted, does not clump group O or B cells at all. It precipitates promptly and specifically with blood group A substance[6] prepared from hog gastric mucin, and the supernatant from a mixture of these two reagents in optimal proportions contains no detectable A or anti-A activity.

We plan to investigate this interesting substance further; we already have some reason to think that this new anti-A agglutinin may prove useful in distinguishing homozygous AA from heterozygous AO erythrocytes[7].

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Differentiation between Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System

Opsonic antibody by combining specifically with antigen facilitates ingestion of that antigen by leucocytes. Recently, a cytophilic antibody has been described which becomes attached to mammalian cells *in vitro* in such a way that antigen is specifically adsorbed[1,2]. The investigation reported here was made to compare the ability of these two types of antibody to potentiate phagocytosis.

Groups of 30 to 40 mice were injected with bovine γ-globulin (BGG) or bovine plasma albumin (BPA) in Freund's complete adjuvant, or *Escherichia coli* somatic polysaccharide antigen. Antisera from each group were pooled. The γ- and β-globulins were precipitated by sodium sulphate[3], purified on DEAE cellulose with a phosphate borate buffer to obtain the γ-globulin[4], and divided into sub-fractions by electrophoresis once or twice in agar gel[5], the extent of the separation in the gel being detected by rabbit anti-mouse globulin.

Each sub-fraction was brought to the same protein concentration of 0·25 mg/ml. They were tested for the following antibody activities:

(1) Agglutination of sheep red cells to which BGG and BPA were attached either by the tanned red cell technique[6] or by the *bis*-diazo benzidine coupling method[7], and to red cells treated directly by the polysaccharide[8].

(2) Sensitization of the skin to anaphylaxis in mice[9] (PCA).

(3) Adsorption of antigen by macrophages treated with cytophilic antibody (CT). The macrophages were in monolayers obtained by washing the peritoneal cavity of a mouse with Hanks's balanced salt solution, and diluting the suspension in Hanks's containing 10 per cent normal mouse serum from which any specific antibody had been removed by adsorption in the cold with red cells coated with the specific antigen by *bis*-diazo benzidine. Monolayers were formed by adding the suspension to glass slides for 1 h at 37° C. After washing they were treated with the antibody preparations for 0·5–1 h, washed twice for 30 min, and exposed for 1 h on a rocker at room temperature to a 0·5 per cent suspension of the antigen-coated red cells. Monolayers treated with *E. coli* antisera were also exposed to a suspension of the heat-killed organisms. After washing, the slides were examined under phase contrast. In a positive reaction large numbers of red cells or organisms became adherent to the macrophages; in a negative reaction nearly all the antigen particles remained evenly dispersed between the macrophages.

(4) Opsonization to phagocytosis. Opsonin was detected by exposing the antigen particles at 37° C to the antibody preparations for 1 h, washing them and adding the sensitized particles to the monolayers maintained at 37° C. The result was read after 0·5 and 1 h. An antibody preparation was considered to contain opsonin when the number of macrophages ingesting antigen was at least twice that in a test of the same antibody preparation adsorbed with the appropriate antigens.

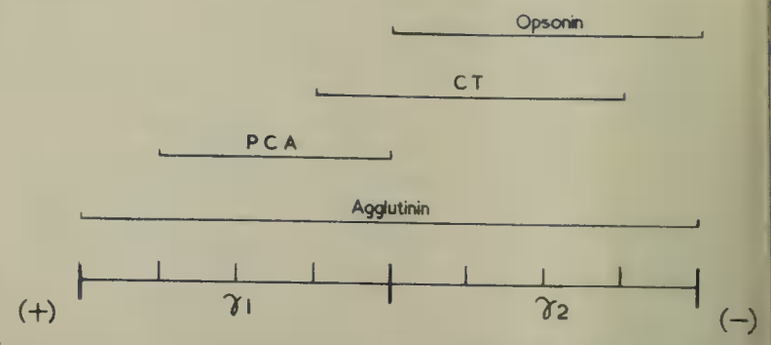

Fig. 1. Antibody activity to protein antigens in fractions of mouse γ-globulin

After each test for CT antibody and opsonin, a duplicate slide of each was treated with normal mouse serum adsorbed in the cold to remove antibody to sheep cells and the specific antigen, and also adsorbed by macrophages to remove non-specific CT antibody. This provided complement-like factors which increased the number of macrophages ingesting antigen in both tests.

Two globulin fractions γ1 and γ2 were separated[10], and each divided into 4 sub-fractions. The various antibody activities to protein antigens in all fractions were presumably those of 7*S* globulins since the potencies of the fractions were unchanged by treatment with 0·09 M mercaptoethanol *p*H 7·0.

All 8 sub-fractions of anti-BGG and anti-BPA contained agglutinins (Fig. 1). Antibody sensitizing skin to passive cutaneous anaphylaxis (PCA) was confined to 3 of the γ1 fractions, two of which contained no cytophilic antibody (CT), thus confirming the report that cytophilic antibody does not sensitize to PCA[2].

Opsonin and CT antibody to BGG and BPA were both present in 3 of the γ2 sub-fractions, but one γ1 sub-fraction contained only CT antibody, and one γ2 sub-fraction only opsonin. It was possible to remove selectively the CT antibody from fractions containing both by adsorption with macrophages or chopped whole mouse spleen, whereas adsorption with antigen-coated particles removed both types of antibody.

CT antibody was detectable only on macrophages. There was no adherence to lymphocytes, polymorphonuclear leucocytes or kidney epithelium, which therefore do not take up CT antibody; even 97 per cent pure suspensions of lymphocytes failed to adsorb it. The uptake of cytophilic antibody by cells separated from whole organs[1,2,11] may be a reflexion of their content of macrophages, though uptake by mast cells has been reported and also red cells[11].

The proportion of macrophages ingesting protein-coated antigen particles in tests with CT antibody and opsonin is recorded in Table 1. More macrophages ingested antigen after treatment with cytophilic antibody than those treated with a similar fraction of normal serum. If serum adsorbed free of specific antigen and unrelated CT antibody was added after the antigen particles had adhered to the macrophages, ingestion was greatly increased.

In summary, the properties of cytophilic antibody specific for the proteins are that it is adsorbed by living

Table 1. Proportion of Macrophages, out of 200, ingesting Protein Antigen coated on Red Cells in Tests with Cytophilic (CT) Antibody and Opsonin

	Macrophages, prior treatment	Test with	Mean % phagocytosis
(1)	Nil	Antigen	20
(2)	Normal serum protein	,,	17
(3)	Nil	(Antigen + CT)	28
(4)	CT antibody	Antigen	58
(5)	Opsonin	,,	19
(6)	Nil	(Antigen + opsonin)	92

macrophages or those gently killed by drying or freezing, but not by lymphocytes or polymorphonuclear leucocytes. Antigen treated with CT antibody, even in antibody excess, is not adsorbed to normal macrophages. Adsorption of CT antibody is blocked by previous treatment of the macrophages with CT antibody of unrelated specificity, and antigen particles adherent to treated washed macrophages may be eluted by addition of serum containing an unrelated CT antibody. Macrophages treated with CT antibody ingest more antigen particles than cells treated with similar fractions of normal serum. On the other hand, opsonin is not adsorbed to macrophages or polymorphonuclear leucocytes, but combines with antigen particles so that large numbers are ingested by both types of cell.

Analysis of *E. coli* antisera yielded a slightly different result. The CT antibody present in the intact serum was apparently inactivated during salt fractionation and separation on DEAE, and it was destroyed by mercaptoethanol. It appears, therefore, to be a 19*S* macroglobulin. It promoted ingestion of killed *E. coli* by the macrophages, but not ingestion of red cells coated with the somatic polysaccharide though these adhered to all the treated macrophages. It was clearly distinct from the opsonin which was stable to the fractionation procedures and unchanged by mercaptoethanol. This is similar to the finding of cell-bound 19*S* antibody and free 7*S* antibody in mice infected with attenuated *Salmonella typhimurium*[12].

The first stage of phagocytosis is contact of the particle with the surface of the phagocyte. Opsonic antibody changes the surface of the antigen particles so that they are more readily ingested by macrophages and polymorphonuclear leucocytes. Cytophilic antibody is a poor opsonin when added to free antigen, but it increases the speed and number of antigen particles becoming adherent to macrophages; as a result some of the particles are ingested. It is possible that *in vivo* cytophilic antibody sensitizes the macrophages lining sinusoids of lymph nodes, spleen, liver or other organs, so that foreign material, for example, bacteria or their toxins, are more rapidly removed from the circulation. Thus its probable function is to act as a 'cell-bound' (or 'cell-fixed') opsonin

I thank Prof. A. A. Miles for his advice.

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BIOLOGY

Signal Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr. (Pisces : Cichlidae)

In some of the mouthbrooding *Tilapia* species, during breeding season the males develop highly conspicuous appendages on their genital papillae. These are long bifid filaments which are either fringed or festooned with colourful blobs of tissue. The biological significance of these 'genital tassels' is still obscure, although field-studies have indicated that they play a part during spawning activities[1,2]. Careful observations of *T. macrochir* in our laboratory tanks have revealed some interesting details. The genital tassel of the male in this species consists of two bright white threads fringed with a few smaller threads of the same colour (Fig. 1). In a fully grown male of 30–40 cm the appendage reaches a length of 5 cm. It acts as a special signal which is presented to the 'ripe' female during courtship and spawning, and assures fertilization of the eggs in the following way:

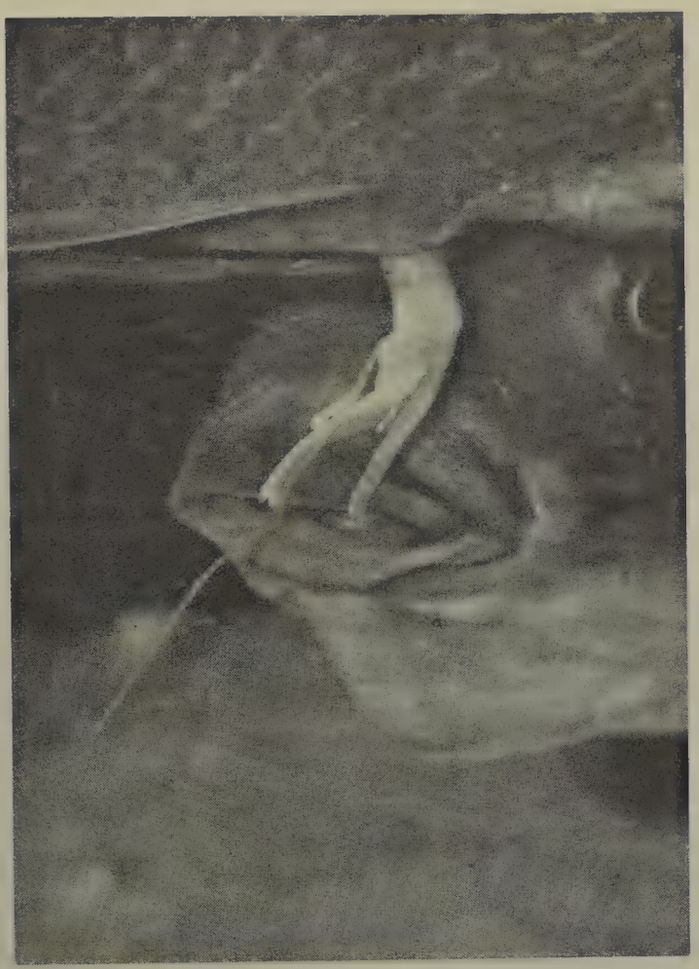

Fig. 1. Female nibbling at the male's genital appendage. The sperm thread is clearly visible at the lower left

(1) When a female is ready to spawn she enters a male's spawning pit. After some courtship movements, the male starts to drag his tassel over the bottom of the pit and up the surrounding wall. On top of the wall, he presses his genital papilla to the ground and then rises and swims tail-first back over the female across the spawning pit (Fig. 2). Simultaneously he ejects from his genital opening a long, 0·5-mm-thick whitish thread which sticks by its free end—for a very short time only—to the spot where he stopped dragging his tassel, and a few seconds later the threads drift away in the water.

(2) The female follows the male and grasps the spermatophore-like thread between her lips and inhales it. Very often she not only takes the thread but also the whole genital tassel, or parts of it (Fig. 1), into her mouth. She does this even before she has started to lay eggs and later fairly regularly following each single spawning act. She repeatedly drops some ten eggs, often from a rather peculiar position, namely, standing obliquely head-down several centimetres over the pit. She does not, however, immediately snap up the eggs into her mouth, but will usually follow the tassel-dragging male once more.

(3) The sperm-thread which the male ejects does not normally meet the eggs immediately, since it drifts away from the pit while the eggs are still laying there. The female, therefore, has to snap up the thread to bring it into contact with the eggs, which she starts to gather into

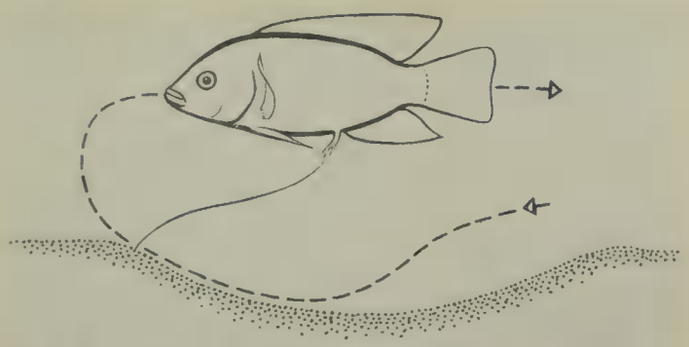

Fig. 2. Movements of the fertilizing male ejecting the sperm thread

her mouth immediately afterwards. The solid sperm mass of this species seems rather favourable in this situation since it is not blown out through the female's gill-openings as readily as would be a loose cloud of milt-fluid found in most of the other *Tilapia* and cichlid-species.

(4) The tassel directs the female's attention to the male's genital opening just before the sperm is ejected, and hence minimizes the danger of the 'spermatophore' being lost. This seems to be the main function of the tassel, which in *T. macrochir* resembles a large number of whitish sperm-threads and, indeed, is treated by the female as such. I hope to investigate this further with dummy experiments.

From the point of view of comparative zoology, it would be interesting to know whether one of the many related species has proceeded still further to true spermatophores. The whole spawning procedure of *T. macrochir* offers phylogenetically important parallels to that of the *Haplochromis*-species in which fertilization of the eggs also takes place within the mouth of the female, and is ensured by egg-dummies painted on the male's anal fin, which are treated by the female as real eggs[3–5].

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Emission of Feulgen-positive Particles during the *in vitro* Maturation of Toad Ovocytes

There has recently been a growing interest in the possible role and localization of cytoplasmic deoxyribonucleic acids (DNA) in amphibian eggs. Our own observations[1–3] suggest that large amphibian ovocytes contain at least two different kinds of DNA: a major constituent, which is bound to the yolk platelets and is probably a reserve material of low molecular weight, and a minor component which surrounds the germinal vesicle of the larger ovocytes: the latter is relatively resistant to acid hydrolysis and might originate from the nuclear sap which, as shown by Izawa *et al.*[4], contains appreciable amounts of DNA. It is of interest to examine the distribution of DNA in ovocytes which, as a result of a treatment with pituitary hormones, undergo maturation (breakdown of the germinal vesicle which results in the mixing of the nuclear sap and the cytoplasm). In fact, some 25 years ago, I examined[5] DNA distribution during the spontaneous maturation of frog (*Rana temporaria*) ovocytes: it was observed that the breakdown of the germinal vesicle is followed by the appearance of small Feulgen-positive granules which lie close to the chromosomes. Their origin and fate could not be established.

More precise observations have now been made on ovocytes the maturation of which had been obtained *in vitro*, following the technique of Dettlaff *et al.*[6]. Large ovocytes of *Bufo bufo* or *Xenopus laevis* were placed in 15 ml. of Ringer's solution containing 4 crushed pituitaries of the same species. They were fixed in Zenker every 15 min; alternate sections were stained with methyl green-pyronine and with the Feulgen reaction. Four experiments were made with *Bufo* and one with *Xenopus*. In all of them but one (*Bufo*), *in vitro* maturation was successful. Two experiments with *Triturus* and one with *Pleurodeles* failed: the breakdown of the germinal vesicle was still incomplete after a treatment with 9 pituitaries for more than one day. Our observations are thus, for the time being, restricted to the Anurans.

In both *Bufo* and *Xenopus*, after a 3-h hormonal treatment, the nuclear membrane shows numerous invaginations. They stain strongly with pyronine and appear on the vegetal side. At the same time, the nuclear sap becomes more hydrated and basophilic. The chromosomes are condensed, while the nucleoli, which have moved towards the animal side of the germinal vesicle, begin to shrink and to loose their basophilia. These changes in ribonucleic acid (RNA) distribution might correspond to the synthesis of small amounts of heterogeneous RNA observed by Brown and Littna[7].

A couple of hours later, the vegetal side of the nuclear membrane is completely broken down; the nuclear sap is now fibrous in appearance and looks like a giant spindle; it carries the strongly Feulgen positive chromosomes. At the limit of the 'spindle', where remnants of the nuclear membrane can still be seen, very small Feulgen-positive particles can be observed. They are never in visible contact with the chromosomes and are very numerous (about 100).

Later on, these fine particles coalesce to form larger spherical Feulgen-positive bodies, which surround the spindle and the chromosomes. Both bodies and spindle move together towards the animal pole. Finally, after a 7–8-h treatment with the pituitary hormones, one or two large bodies, which stain intensely with Feulgen, can be observed in the cortex of the ovocyte, at a short distance from the maturation figure (Fig. 1). Judging from the intensity of the Feulgen reaction (which is negative after treatment of the sections with deoxyribonuclease), these cortical bodies must contain much more DNA than the chromosomes.

The origin and fate of these cortical Feulgen-positive spheres (which can no longer be seen in ripe unfertilized eggs) will remain enigmatic so long as combined autoradiographic experiments (incorporation of thymidine) and electron microscopy observations have not been performed. Although it is impossible for the time being to exclude a chromosomal or nucleclar origin, the most likely interpretation of our observations is that the

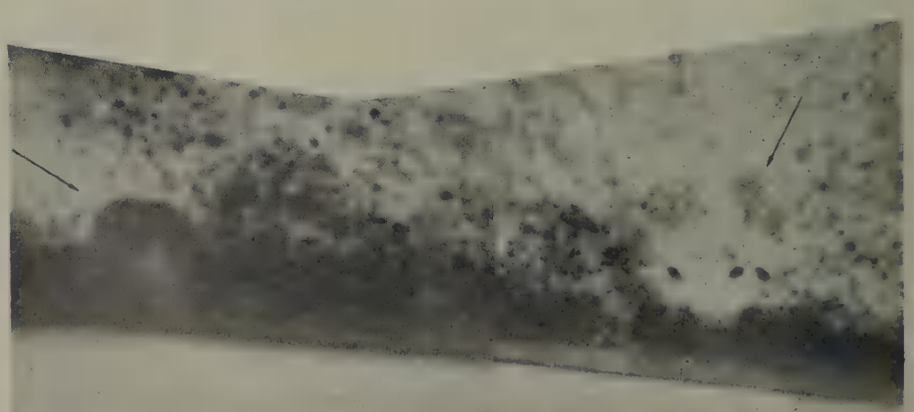

Fig. 1. Cortex of a *Bufo bufo* ovocyte undergoing *in vitro* maturation after 7 h. Arrow on the left, maturation spindle and chromosomes; arrow on the right, Feulgen-positive sphere

Feulgen-positive granules and spheres arise from the nuclear sap, when it mixes with the cytoplasm. If so, they would have the same origin as the DNA found around the germinal vesicle in very large *Triturus* ovocytes[2].

It can be concluded from these observations that, in all anuran species examined (*Rana*, *Bufo*, *Xenopus*), mixing of the nuclear sap with the cytoplasm, as a result of an *in vivo* or *in vitro* hormonal treatment, leads to the production of extra-nuclear DNA, which ultimately moves into the cortex of the egg. This cortical localization of the Feulgen-positive spheres might be very significant, since we know the extreme importance of the dorsal cortex (grey crescent) for morphogenesis (gastrulation, neural induction)[8]. As I have pointed out recently[9], the dorsal cortex of the fertilized egg must, ultimately, control the activation (or de-repression) of certain genes in the dorsal lip of the blastoporus (organizer). It is conceivable that this de-repression might be mediated, as suggested recently by Frenster[10], by a DNA-like RNA, which should be, in the present case, synthesized on this cytoplasmic DNA template. Very recent experiments by Curtis[11] suggest that the dorsal cortex of *Xenopus* eggs possesses its own heredity: his results can only be understood if one assumes that it contains particles capable of independent replication. The presence of DNA, originating from nucleocytoplasmic interactions during maturation, in the cortex might perhaps account for these curious observations. Another attractive possibility is that the cortical DNA might play a part in the determination of the germ-cells, since it is known, from the work of Blackler[12], that ultraviolet irradiation of the vegetal pole in fertilized eggs results in sterility. Much more work is obviously needed before the function of the 'cortical DNA' becomes clear.

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Wheel-running Preferences of *Peromyscus*

The bases for the strong predilection of captive and domestic rodents for wheel-running are not fully understood. It is clear that wheel-running provides a relatively strenuous form of muscular exercise which is not otherwise available to confined animals. In addition, the acts of manipulation and control of the movements of an activity wheel may be rewarding to rodents, quite independently of exercise[1]. Rodents also find it rewarding to run motor-driven activity wheels which they start and stop by pressing levers[1].

That the attractiveness of wheel-running cannot be attributed solely to the fact that it is an outlet for vigorous muscular activity is shown by the finding that rodents in extensive enclosures which contain complex burrow-simulating mazes and hundreds of feet of linear runways frequently spend much of their time running an activity wheel[2]. The present study grew out of the observation that deer mice also learn to run activity wheels shaped into squares, triangles, ellipses, etc., even when round wheels are also available[3].

A $15 \times 15 \times 14$ in. enclosure was constructed of 'Perspex' and hardware-cloth, containing four adjacent coaxial activity wheels made of 3-in. strips of 1/8-in. mesh hardware-cloth. Dual ball-bearing mountings gave all wheels a very low frictional torque. The 'round smooth' and 'large round' wheels were 10 in. in diameter, the former containing an inner lining of acetate film. The 'small round' wheel was 6 in. in diameter, and the 'square' wheel was 7 in. square. The highest rates at which these wheels were run were: smooth round, 84; large round, 90; small round, 138; and square, 114 r.p.m. Wheel revolutions were sensed by a microswitch actuated by $\frac{1}{4}$-in. ball bearings on the end of a half-inch metal strip forming a spoke of the wheel. The spokes extended through slots in the 'Perspex' roof of the enclosure, on which the microswitches were mounted.

Two litters of young captive-born canyon mice, *Peromyscus crinitus* (litter 1, 8 weeks old, 2♀, 1♂; litter 2, 7 weeks old, 3♂), and 2 old males, captured 3 years previously, were selected for study. The old animals had had experience running motor-driven and freely turning wheels $2\frac{1}{2}$ years earlier (see ref. 1). The old males were studied individually, whereas the 3 animals in each litter were studied in consort.

Simply making activity wheels available to inexperienced *Peromyscus*, even for several weeks, does not necessarily give information concerning the preferences of experienced animals for the different types of wheels, because *Peromyscus* generally tend to be conservative in their behaviour[3]. Thus, whichever wheel an animal learns to run first often monopolizes its subsequent activity—the other wheels virtually being ignored. The following programmes were adopted to ensure that the animals would become experienced in running all the wheels. Using individual males, all 4 wheels were made available at first (phase 1) until a constant pattern of wheel running was established and maintained for 2–4 days. The wheel in which the most revolutions were run (which generally was the one in which the most time was spent running) was then locked, and the experiment was continued with 3 wheels unlocked (phase 2) until a new constant pattern was established and maintained for 2–4 days. The favoured wheel of the 3 was then locked (phase 3) and, afterwards, the favoured wheel of the remaining 2 (phase 4). After the animal had learned to run the last unlocked wheel, the wheel of third choice was unlocked until a constant pattern of running was established and maintained for 2–4 days (phase 5). The wheel of second choice was then unlocked (phase 6) and, finally, the wheel originally of first choice was unlocked (phase 7). Phase 7, in which all 4 wheels were available to the experienced animal, lasted at least 6 days. The programme with groups of 3 young animals in consort differed solely in that only 2 wheels were locked (that is, phases 4 and 5, being unnecessary, were omitted).

Running comparisons were made on the basis of average numbers of revolutions run in the different wheels per night. Time spent running, although not measured, is roughly proportional to the product of the number of revolutions run and the circumference of the wheel (7π for the square wheel). The values plotted in Fig. 1 are the averages for the adapted running of the last 2 or more days of each phase. A bright-dim light cycle (L:D 14:10) was used. Recording methods and other experimental conditions have been described elsewhere[1,2].

In phase 1, animal 20 ran the large round wheel almost exclusively (13,202 revolutions). When this wheel was locked it preferred the round smooth wheel (21,284 revolutions). In phase 3 it ran 28,679 revolutions in the square wheel and only 5,610 in the small round wheel (Fig. 1). With only the small round wheel unlocked (phase 4) it ran 43,051 revolutions. When the square wheel was unlocked in phase 5 the animal resumed its preference for it (38,905 compared with 7,167 revolutions) and maintained this

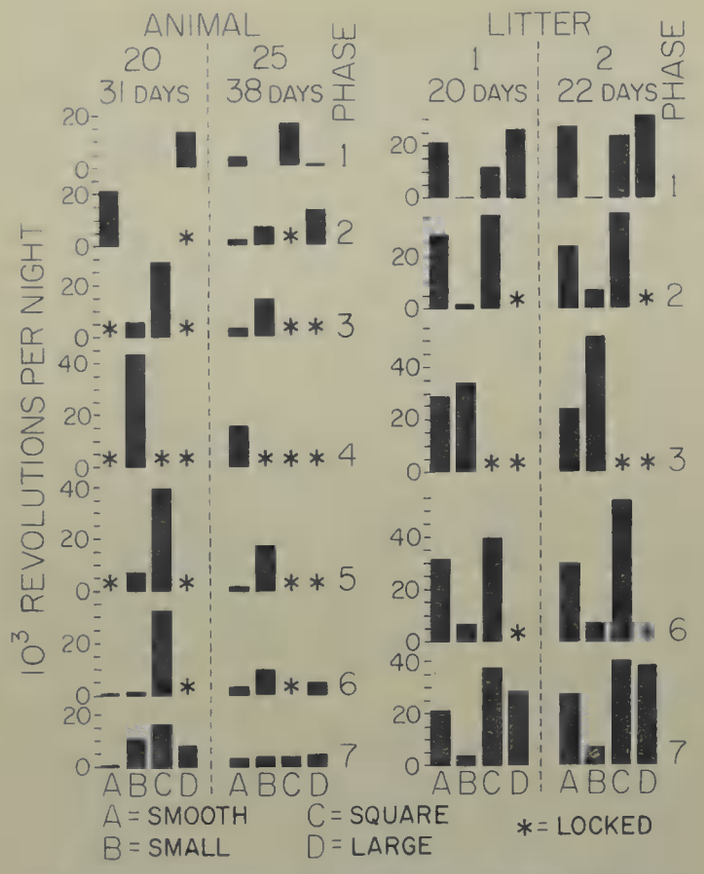

Fig. 1. Wheel running by canyon mice during the programmes described in the text. The figures for days at the top of each data plot refer to the total durations of the programmes

preference in phases 6 and 7. Thus, although the square and small round wheels were not run in phases 1 and 2, they became the wheels of preference in subsequent phases, with the square wheel very much favoured in phases 5 and 6 (Fig. 1). Animal 25 ran much less than animal 20. It preferred the square wheel in phase 1, the large round wheel in phase 2, and the small round wheel in phase 3. It returned to the small round wheel in phase 5, maintained this preference in phase 6 and showed a slight preference for the large round wheel in phase 7.

The 2 groups of young litter mates showed identical sequences of wheel-running preferences (Fig. 1). In phase 1, the order of preference was: large round, smooth round, and square; the small round wheel was more or less ignored. In phase 2 the square wheel was preferred, with some running of the small round one. In phase 3, the small round wheel was preferred to the smooth round one. The preference for the square wheel persisted when it was unlocked (phase 6), but the smooth round wheel now was run much more than the small round wheel. Finally, phase 7 saw a return to running the large round wheel, which had been preferred in phase 1, but the square wheel remained the preferred wheel.

The results of this exploratory study suggest that: (1) although canyon mice may not run certain activity wheels at all when several types are available, none the less they can learn to run these wheels with great facility; (2) the preferences that are established by giving inexperienced animals a choice of several different types of activity wheels usually differ from those of the same animals after they have had experience in running all of the wheels; (3) a wheel with a hardware-cloth surface is preferred to one with a smooth surface; and (4) experienced animals may prefer a square wheel to a round one.

The implication that can be drawn tentatively from the generally slight to marked preference of experienced animals for a square wheel is that confined wild rodents tend to select forms of exercise that require quick reflex actions and split-second timing and co-ordination of movements (running the square wheel required jumping at the corners at rates up to 9 times per second) in preference to forms in which vigorous muscular activity is the primary requirement. In view of these findings, it appears that round activity wheels may not be the most suitable type for use in studies of confined wild rodents. Experiments designed to explore these implications further with larger numbers of animals of several species are in progress.

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Methylcholanthrene Carcinogenesis influenced by Vegetable Extracts

A group of 700 three-month-old male mice received subcutaneous injections of 0·1 mg methylcholanthrene in benzene and were then observed for the appearance of tumours for 120 days. The injected mice were divided into four groups which were fed differently.

Group I, the controls, were fed on our standard ration of oats added to maize groats boiled in milk, to which cod liver oil, linseed oil and a polyvitamin preparation were added.

Group II received no raw vegetable food from the day of birth. In place of oats they received bread boiled in milk; their litter of beechwood shavings was sterilized in the autoclave and the complete destruction of enzyme activity in food and litter was confirmed by testing for catalase, which was always absent.

Group III were treated similarly except that they were deprived of vegetable food only from the day of injection.

Fig. 1. Symptom of virosis on leaves of *Nicotiana samsun* (light green mosaic) after treatment with aqueous-extract of oats and the seeds of its usual weeds (*Vicia* sp., *Brassica rapa*, *Agrostemma githago*, *Cirsium* sp.)

Fig. 2. Symptom of virosis (*malformation*) on the leaves of hybrid tomato provoked with aqueous extract of oats and the seeds of its usual weeds (*Vicia* sp., *Brassica rapa*, *Agrostemma githago*, *Cirsium* sp.)

Group IV were fed like Group I, but given subcutaneous injections of 0·1 ml. 'vegetable extract' twice weekly for six weeks.

The 'vegetable extract' was prepared as follows: 10 g oats was ground to powder, and 100 ml. distilled water added. After standing 1–2 h it was centrifuged for 1 h at 4,000 r.p.m., and the supernatant filtered through a Seitz *EK* filter. This extract contained a number of plant viruses, since on testing in tobacco, tomatoes and beans characteristic changes occurred (Figs. 1 and 2).

Table 1. Effect of Fresh and Autoclaved Vegetables on Frequency of Methylcholanthrene-induced Tumours in Mice

Group	No. of animals at start of experiment	No. of animals developing tumours Expected	Observed	No. of animals surviving to 120 days without tumours	Total No. of animals completing experiment	χ^2
I	300	(95·83)	108	192	300	1·37
II	100	(31·62)	11	88	99	39·5
III	150	(46·95)	27	120	147	14·7
IV	150	(47·60)	76	73	149	31·0
Total	700		222	473	695	86·57

Total $\chi^2 = 86·57$. $P \ll 0·001$.

The results are given in Table 1. It is clear that this vegetable extract enhances, whereas a diet free from raw vegetable materials depresses, the carcinogenic activity of methylcholanthrene. Is it the plant viruses which are the cause of this difference?

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ANATOMY

Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear

It has been found that when wounds are made through the full-thickness of the pinna of the rabbit's ear, and tissue of area 1 cm² is excised (Fig. 1), the tissue deficit is almost completely made good within 98 days, though a small hole always remains (Fig. 2). This entails the regeneration of relatively mobile dorsal skin, elastic cartilage, connective tissue, nerve fibres and immobile

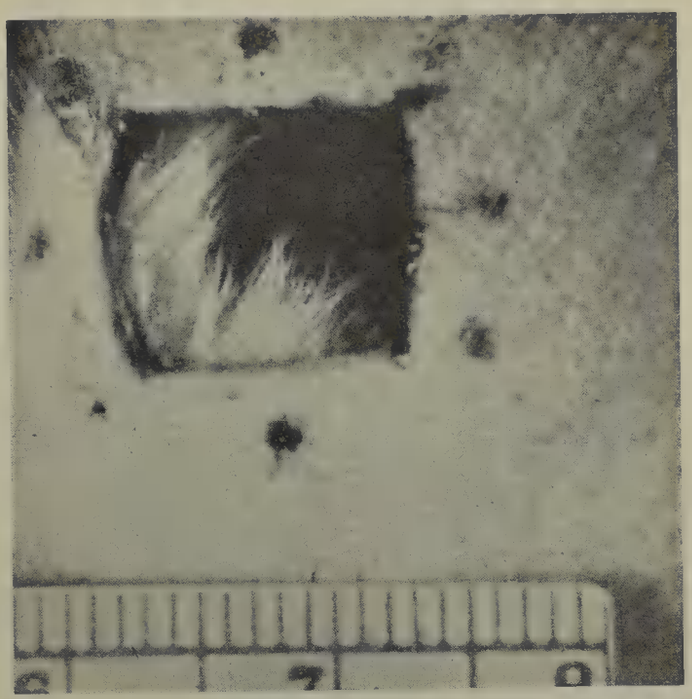

Fig. 1. Appearance of the inside of the ear following removal of a full-thickness portion 1 cm × 1 cm in area

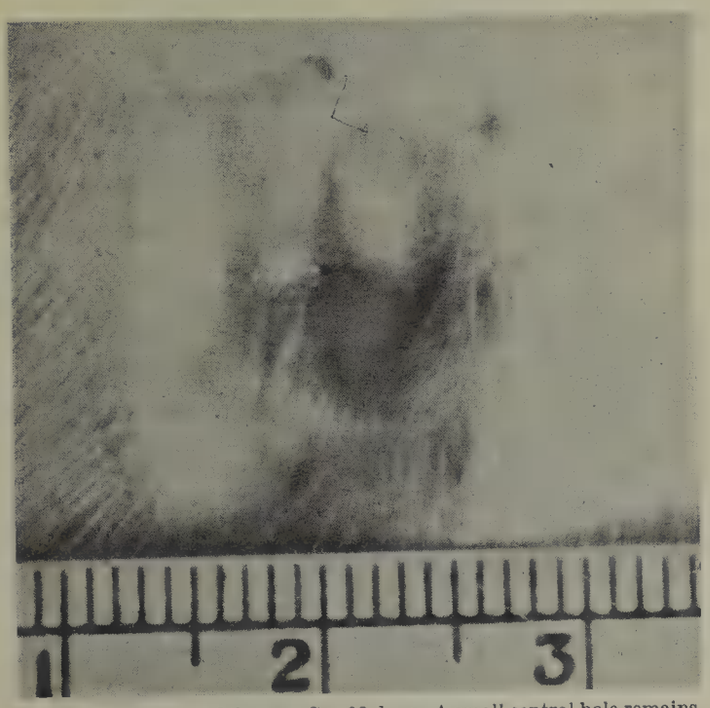

Fig. 2. Appearance of the ear after 98 days. A small central hole remains, but the deficit has otherwise been made good

ventral skin. Furthermore, the process takes place significantly faster in males than in females. It has been reported elsewhere that if either skin[1] or cartilage[2] is removed from the rabbit's ear it regenerates, the skin completely and the cartilage partially. The replacement of full-thickness deficits has not, however, been reported.

Tissue of area 1 cm² was removed with a specially designed punch from the ears of 30 rabbits (18 male and 12 female) under 'Nembutal' anaesthesia. Care was taken to avoid major blood vessels. Photographic records were kept of the progress of regeneration, the ears being photographed every 7 days. The areas of (*a*) the central hole, and (*b*) the zone bordered by the old ventral skin, were calculated from projections of the transparencies on to graph paper by outlining the areas and counting the enclosed squares. The area of the new tissue (a measure of the amount of growth) was determined by subtracting (*a*) from (*b*). The amount of contraction (the process whereby the size of the initial deficit is altered by movements of the old adjacent skin) was obtained by subtracting (*b*) from the initial deficit area. All measure-

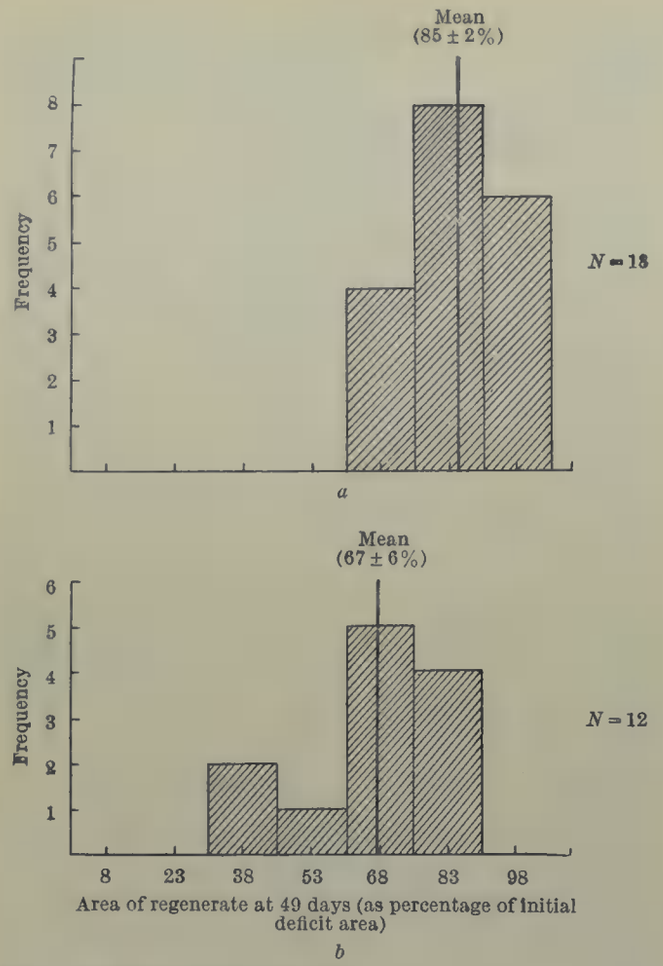

Fig. 3. Histograms showing variation in growth of the regenerate in male (*a*) and female (*b*) animals

ments of growth and contraction were expressed as percentages of the initial deficit area.

By 49 days there was a difference of 18 per cent between the areas of the regenerate in males (85 ± 2 per cent) and in females (67 ± 6 per cent); this difference was found to be highly significant ($P < 0.005$). The data on which this was based are given in Table 1 and illustrated in Fig. 3. The difference between contraction in males (3 ± 3 per cent) and in females (1 ± 5 per cent) was not found to be significant ($P > 0.3$). It is possible that the regeneration

Table 1. Variation in Growth of the Regenerate in Males and Females

	Area of initial deficit (cm²)	Area bordered by old skin at 49 days (cm²)	Area of hole at 49 days (cm²)	Area of regenerate as % of initial deficit
♂	0·95	0·87	0·02	90
	1·03	1·06	0	103
	1·00	0·94	0·01	93
	0·99	1·08	0·29	80
	1·12	1·12	0·18	84
	1·01	0·88	0·05	82
	1·05	0·99	0·28	68
	1·10	0·83	0	75
	0·98	0·76	0·10	67
	0·99	0·95	0·02	94
	0·95	1·09	0·30	83
	0·98	1·17	0·29	90
	1·02	1·08	0·27	79
	0·98	1·00	0·06	96
	1·02	1·07	0·02	103
	1·05	0·94	0·07	83
	1·02	0·99	0·04	93
	1·10	0·84	0·07	70
			($N = 18$, $x = 85 \pm 2\%$)	
♀	1·11	1·22	0·43	71
	1·08	1·18	0·38	74
	1·07	1·05	0·60	42
	1·02	0·92	0·57	34
	0·97	0·92	0·13	81
	1·00	1·03	0·18	85
	1·01	1·10	0·45	64
	0·99	1·01	0·15	87
	1·09	1·16	0·63	48
	1·05	1·30	0·44	82
	1·15	0·78	0·02	66
	1·03	0·76	0	73
			($N = 12$, $x = 67 \pm 6\%$)	

rate is influenced by androgens. Although in large doses they inhibit the production of granulation tissue[3,4], most indications are that they promote reparative processes[5–7]. This may explain the faster rate found in males. Investigations into this possibility are now in progress.

The rabbit's ear provides a useful material for the investigation of mammalian tissue regeneration. Its flatness and position facilitate the keeping of photographic records of the progress of regeneration from which one parameter of this process, surface area, can be measured accurately. It is thus possible to make quantitative observations of mammalian tissue regeneration over a period of time without causing further trauma which would naturally interfere with the process. It also provides an opportunity to study quantitatively the effects of mechanical and metabolic interference on mammalian regeneration at the tissue level.

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Possible Role of Neuroglia

In a recent communication[1], Hertz has postulated an impulse transmission system in which "potassium ions, which have been lost from one nerve cell during its activity, are transported through neuroglia cells to the outer surface of another nerve cell, which is then depolarized and stimulated; that is, a neuronal–neuroglial–neuronal impulse system". For such a system to operate, the neuroglia cells should bear an appropriate topographical relationship to the nerve cells and should have a capacity for active ion transport; for the pathways to be selective there should be no significant diffusion of potassium ions between cells. Hertz has given good supporting evidence for the second premise, the capacity of glia cells for active ion transport; but the first and third require examination.

The direct synaptic connexion between nerve cells and glia cells which Hertz mentions was described by Scheibel and Scheibel on the basis of Golgi preparations[2]. While these techniques retain an important role in the mapping of neuronal processes, they are no longer acceptable for the demonstration of intimate neural contacts and their interpretation is questionable. Moreover, the synapse-like contacts the Scheibels described were limited to "the application of axonal *boutons en passage* and *boutons terminaux* to the cytoplasm and/or nucleus of the oligodendrocyte". Such limited contacts, if they exist, are not highly relevant to a general theory of impulse transmission. More relevant are certain observations by electron microscopists. When adjacent cells come together the outer limits of their osmiophilic plasma membranes usually remain separated by an interval of 100–150 Å. In certain situations this interval closes with fusion between the surface dark layers of the adjoining cells. Closed contacts in the central nervous system are formed between glia cells and processes and between endothelial cells[3] but have not been observed between neurones and neuroglia. Furthermore, astrocytic processes are arranged in such a way that they isolate the receptive surfaces of

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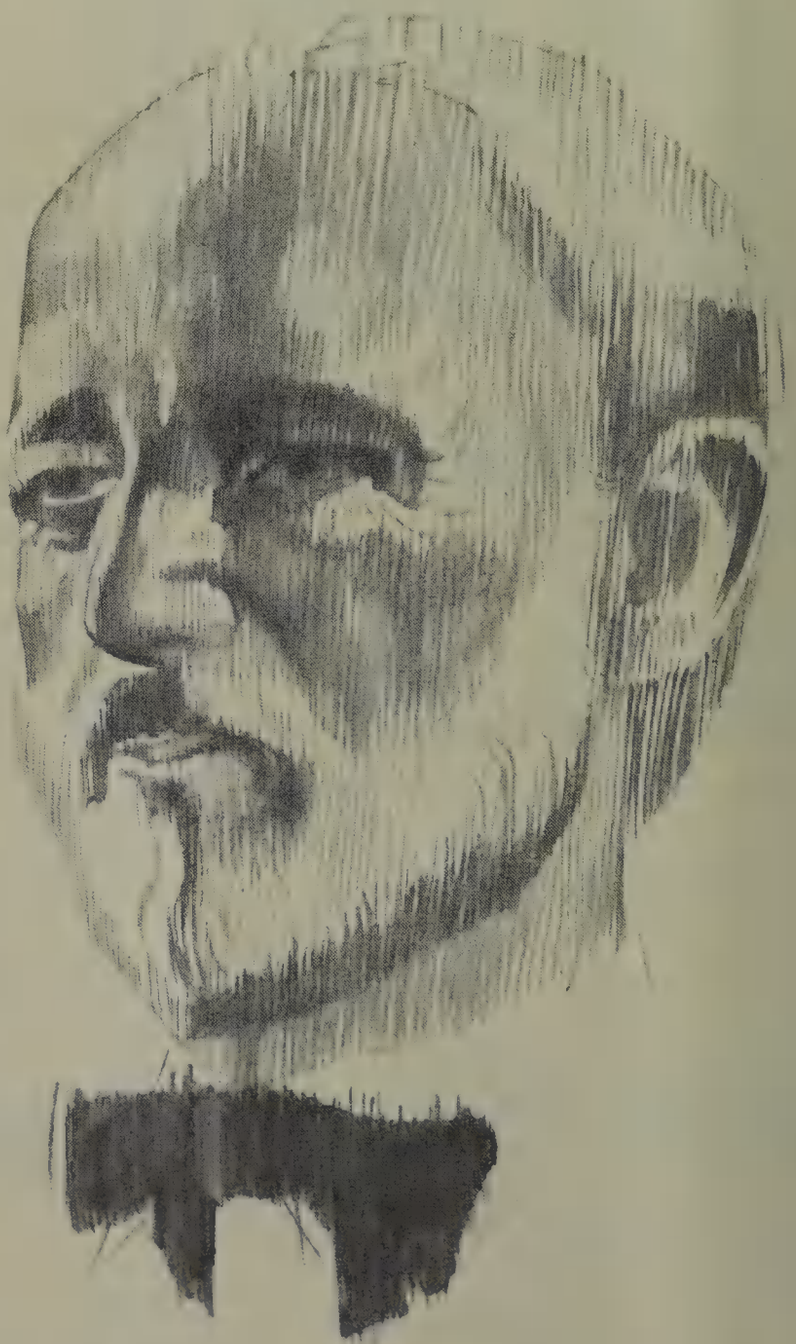

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neurones from all axon terminals except those that synapse specifically on them[4]. Since neuroglia–neuronal contacts are open, there exists a perineuronal space of 100–150 Å; this may be contained by closed contacts between glia cells and, as will be seen, is available for diffusion. While neuroglia–neuronal contacts are open and apposed plasma membranes are not thickened, the contacts are not unspecialized. Where certain glial processes abut against neuronal perikarya or dendrites, there is often a sub-surface cistern beneath the neuronal plasma membrane[5]. The sub-surface cistern may have a mitochondrion closely applied to the inner surface[6]. Taken with the presence of an Na^+—K^+ stimulated ATPase at the outside of the glia cell membrane[7] and the observation that cortical neuroglia reacts to an increase in potassium concentration by an increase in oxygen uptake[1], these observations suggest that certain glia cells may be concerned with the control of the ionic content of the perineuronal intercellular space. Such a control system is necessary to prevent continuous depolarization and spread between neighbouring synaptic areas and along the axonal membrane; the possibility of interference between excitatory and inhibitory synapses here is obvious. Potassium ions are lost to the cell not only during the falling phase of the spike potential during impulse transmission but also during the production of the excitatory post-synaptic potential and particularly during the production of the inhibitory post-synaptic potential[8]. It is suggested that, particularly in the cerebral cortex, excess potassium ions from these sources may be mopped up by active transport into glia cells. It is further suggested that under physiological conditions the potassium ions would be restored to the neurones, perhaps by active transfer via the sub-surface cisterns with an apposed mitochondrion providing the energy to drive the ionic pump. Clearly such a system could be overloaded but, given a low-resistance bypass, short-term overloading is likely to result in a damming up of potassium ions[9] and diversion at the input stage rather than in overflow at the output stage.

Such a low resistance pathway is provided by the intercellular spaces, which are not, however, restricted to those parts of the central nervous system where potassium-induced stimulation of respiration has been demonstrated. In this context the volume of the extracellular space is an irrelevancy. What matters is not the volume (only 5–7 per cent of the total tissue volume[10]) but the arrangement of the intercellular spaces and their effective width. It has been argued that the osmiophilic lines on electronmicrographs do not represent the full thickness of the plasma membrane and that unstained outer layers keep them a uniform distance apart. However, as has been mentioned, the osmiophilic lines can fuse and the bulk of evidence favours the view that the lines represent essentially the full thickness of the membranes[11]. Diffusion through narrow clefts is not free and a theoretical formula has been used to estimate the restriction they impose[12]:

$$\frac{D'}{D} = \frac{(1 - a/r)^2}{1 + 2{\cdot}4\,a/r}$$

where D'/D is the ratio of restricted to free coefficients of diffusion, a is the radius of the particle and r is the radius of the channel. On this basis, the restriction to potassium ions (hydrated radius 1·25 Å) diffusing through a channel of 125 Å diameter would not be great ($D'/D = 0{\cdot}92$). However, this formula probably overestimates the restriction since it includes a correction for steric effects at the entrance to the channel which does not apply and presumes a cylindrical channel of radius r instead of a linear slit of half-width r. Manegold has demonstrated experimentally that, on the average, the slit half-width needs to be reduced to 15 times the particle radius if diffusion is to be restricted by 50 per cent[13]. On this basis, again, the restriction to the diffusion of potassium ions through 100–150 Å intercellular spaces should be slight. More significant than either of these approaches is the demonstration that spaces of these electronmicroscopic dimensions in the central nervous system of the leech provide a rapid and effective pathway for the movement of ions and small molecules[12].

Since this primarily perineuronal pathway has been shown to exist and to be available for diffusion, it is unnecessary to involve the neuroglia as an active intermediary in Leão's spreading depression[1]. According to the neuronal-contiguous theory of its transmission, potassium or a special substance which may be glutamic acid is released from depressed neurones to excite nearby neurones[14]. One need not look beyond the intercellular space to route this spread or to explain the source of the water and ions which enter the apical dendrites[15]. Certainly the advance of spreading depression (2–6 mm/min) is too fast to be accounted for solely by diffusion and too slow for orthodox nervous conduction alone, but this is no argument against a combination of these processes in a neurone–space–neurone sequence of spread. This perhaps operates when the potassium mopping-up mechanism of the neuroglia is overloaded. An important objection to a theory involving neuroglia in the sequence is the limitation of spread from one type of cortex to another. Thus, depression does not spread from neocortex to cingulate mesocortex or to the hippocampus or vice versa[14]. In these situations the neurones are discontiguous and spread fails to occur although the neuroglia cells are contiguous.

Finally, the possibility and time-scale of diffusion of potassium ions through intercellular spaces militates against the suggestion[1] "that the early stages of memory should be related to changes in ionic distribution". Although the long-lasting type of memory trace seems to be characterized by RNA synthesis, with changes in the base composition, in neurones[16] and neuroglia[17], the early stages, which occur within a few hours of training, may be unaffected by RNA inhibitors[18]. Some intermediary process of longer duration must follow the short-term ion changes. Perhaps this includes the induction of ion-stimulated enzymes in response to overloading of a potassium mopping-up and restoration mechanism centred on the neuroglia.

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I AGREE with Drs. Wendell-Smith and Blunt that three pre-requisites of the suggested neuronal–neuroglial–neuronal impulse transmission system are: (1) an appropriate topographical relationship between nerve cells and neuroglia; (2) a high capacity for ion transport in glia cells; (3) no significant intercellular diffusion. Since

Wendell-Smith and Blunt regard my supporting evidence for the second of these premises as good, only the two others will be discussed here.

As regards the topographical relationships, no direct synaptic connexion between nerve cells and glia cells is envisaged in the postulated impulse transfer system. The reference to Scheibel and Scheibel's findings was made to emphasize that the idea of a co-operation between neurones and neuroglia in brain function is not a new one, but it was clearly stated that "participation of neuroglia in the transfer of impulses does not . . . necessarily require any direct synaptic contact between nerve cells and glia cells". The mechanism suggested by me represents another, totally independent, way of transferring impulses between the two cell types; a direct synaptic contact is meaningless in this system and the evidence against the findings by the Scheibels accordingly irrelevant in this context.

In the initial phase of the suggested potassium-mediated transfer of impulses "potassium ions lost from a neurone during excitation are transported actively from outside it into glia cells". This is consistent with the widely accepted presence of narrow (100–150 Å) intercellular channels surrounding the cells, and it is important that the volume available for diffusion is small and the routes of diffusion long and complex. The release of potassium ions may therefore cause a temporary increase in the local concentration of potassium, which in turn stimulates the active transport of potassium ions into glia cells.

Admittedly, some diffusion may occur along the intercellular clefts. The extent of this diffusion in mammalian brain cannot possibly be estimated from measurements on the leech central nervous system, which histologically differs grossly from the vertebrate brain[1,2]; and the findings by Nicholls and Kuffler[3] that, for example, sodium ions do not diffuse into certain types of leech glia cells cannot be universally valid, since the sodium space of brain is much larger than the true extracellular space[4]. It is furthermore of no importance that some potassium ions may become passively distributed in the extracellular space—if only the potassium concentration here does not increase so much that a general excitation of nerve cells occurs. What matters is, whether the active transport is large enough to secure that a sufficient fraction of the released ions are transported via predetermined, regulated pathways to exert a selective action on certain nerve cells. To which degree this requirement is fulfilled cannot be estimated by regarding only the possibilities for diffusion but requires knowledge of the efficiency and rate of active transport. No such information is available. It seems, however, thought-provoking that the extracellular fluid in brain cortex is restricted to narrow clefts, and that the extracellular space is small. In this way the active, regulated transport is probably given a possibility to compete effectively with free diffusion. Wendell-Smith and Blunt seem to agree on this point of view, since they suggest that "excess potassium ions may be mopped up by active transport into glia cells".

The fate of the 'mopped-up' potassium ions will depend on the anatomical inter-relationship between adjacent glia cells and thus the possibilities of active transport from one cell directly into another as well as from the 'terminal' glia cell to the perineuronal cleft. The fusion between surface layers of adjoining glia cells referred to by Wendell-Smith and Blunt may constitute the anatomical basis for such a transport.

The hypothesis of a potassium transport through glia cells was primarily provoked by the biochemical, topographical, developmental, and pharmacological analogies between the potassium-induced stimulation of oxygen uptake and spreading depression. It is a result of an effort to combine neurochemical, neuroanatomical and neurophysiological evidence and experimentally less well founded than the initial uptake of potassium ions by glia cells.

I admit it is "unnecessary to involve the neuroglia as an active intermediary in Leão's spreading depression", but the concept that neurones behave like pools of potassium ions which are released by potassium and in turn transported actively by glia cells to propagate the response fits with the evidence mentioned and gives a simple explanation of the rate of the spread. The massive, self-propagating character of the response is probably due to the initial excitation of a very large number of neurones. The subsequent release of potassium ions must accordingly also be large, and they are transported in all directions; the absence of either cell type must, of course, inhibit the propagation. By the term 'overloading' is understood that the potassium release is "so intense that a certain time is required for its uptake in neuroglia"; this is the reason that neurophysiologically a depression is observed instead of an activation and consistent with the concept by Wendell-Smith and Blunt: "overloading is likely to result in a damming up and diversion at the input stage". Altogether, I therefore find that their concepts differ only slightly from mine, and this may perhaps be taken as an indication that the proposed mechanism is at no variance with present neuroanatomical thinking.

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ENTOMOLOGY

An Improved Method for obtaining, *in vitro*, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae)

The first report on the cultivation of tick (Ixodidae) tissues *in vitro* was that of Řeháček[1] in Czechoslovakia, who obtained proliferation of fibroblast type cells in explants from developing adults of *Dermacentor marginatus*. Řeháček[2,3] afterwards improved his techniques and was able to obtain cell monolayers by trypsinizing tissues of developing adults of *D. marginatus*, *D. pictus* and *Hyalomma dromedarii* and growing them in test-tubes and Carrol flasks. His methods for preparing tick tissues and of culturing them are fully described elsewhere[4].

In culturing tissues from developing adults of *H. dromedarii*, we found that certain modifications in Řeháček's methods and of his medium produced better results. There was improved dissociation of the tissues and hence a higher cell yield when the tissues were agitated by a magnetic stirrer in 0·25 per cent trypsin which had been prewarmed in a 37° C water bath. The resulting cell suspension was filtered through sterile gauze to remove large pieces of undissociated tissue.

The *p*H of the medium was adjusted to 6·9, 0·2–0·4 higher than in Řeháček's experiments, and this appeared to produce more rapid attachment of the cells to the glass. At 27°–29° C, adherence of cells to the glass surface started in less than 20 min and, by 18–20 h, cell attachment was nearly complete. In our earlier trials, we used Leighton tubes with and without cover-glasses and incubated them horizontal. In these tubes, there was a tendency for the cells, over the major part of the glass surface, to aggregate in clumps from which fibroblast type cells grew out, but they never formed a continuous sheet. Although towards the bottom part of the tubes there was a narrow strip where the growing cells had formed a more or less continuous sheet, growth of the culture as a whole was by no means as satisfactory as the uniform cell monolayers obtained in vertebrate cell cultures. Mitoses in the Leighton tube cultures were seen up to 10 days, the same period as in Řeháček's cultures[4].

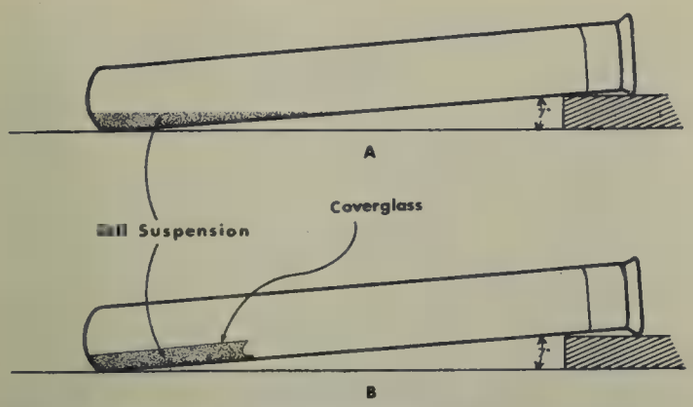

Fig. 1. Cultivation of tick cells in test-tubes, without cover-glass (*A*) and with raised cover-glass (*B*)

In subsequent investigations to improve the cultures, we used 'Pyrex' glass test-tubes 125 mm × 16 mm, incubated at an angle of about 7°, and found that, as in the Leighton tubes, by about the 4th day, there was a patch of cells growing into a sheet towards the bottom of the tubes, but over the rest of the glass surface the cells formed scattered aggregates (Fig. 2, *B*, *D*). We next introduced into the test-tubes, before seeding of cells, rectangular cover-glasses (40 mm × 11 mm) primarily with the object of obtaining growing cells on the cover-glasses for microscopical examination. However, the width of the cover-glass was such that it remained raised above the curved side of the tube (Fig. 1, *B*) with the cell suspension underneath it. In such tubes the cells always grew out on the glass surface of the tube as an excellent, uniform monolayer sheet, consisting mostly of epithelial type cells. There were no cells, or only a few, attached to the cover-glass. In appearance, these cell sheets (Fig. 2, *A*, *C*) were similar to those obtained from vertebrate cells. Furthermore, the metabolism in the tubes appeared to be higher with cover-glasses than without as evident from the more rapid change in the *p*H of the covered medium. The cell sheets in the tubes with cover-glasses have, with periodic changes of medium, been kept for up to 35 days without any obvious signs of degeneration. There was recurring mitotic activity and mitoses were common even 30 days after seeding, the longest period recorded so far for tick tissues *in vitro*.

What caused and maintained the better growth in the tubes with cover-glasses in these preliminary investigations is not clear. Cell aggregation which occurred by about the 4th day in tubes without cover-glasses would appear to slow down metabolism. One explanation may be that the partially closed system of the cell suspension in medium trapped between the overlying cover-glass and the curvature of the tube produces physical forces which prevent the cells from aggregating. Towards the free surfaces of the medium, that is, at the end and less so at the edges of the cover-glass where these forces may be less, the cells do tend to form clumps, particularly at the open end (Fig. 2, *A*, *C*). It is also possible that this confined system produces, except towards the limits, partially anaerobic conditions which may be more suitable for the continued growth of the cells, or that the carbon dioxide evolved during the metabolic processes is prevented from diffusing out to any great extent, thus increasing the carbon dioxide tension of the medium. These aspects are now under investigation. But, whatever the explanation may prove to be, inclusion of an overlying cover-glass appears to maintain consistently better cell growth and a more uniform cell monolayer sheet than if it is omitted. This modification of technique may also enhance the utility of the cell cultures for the cultivation of viruses and other pathogenic organisms, the purpose for which the technique is primarily intended.

We thank Dr. J. Řeháček of the Institute of Virology, Bratislava, Czechoslovakia, for his help at the beginning of these experiments, also Prof. D. S. Bertram, director of this Department, and Prof. Forrest Fulton of the Department of Bacteriology and Immunology for their advice, Mr. D. F. King for staining the tubes, Mr. C. J. Webb for taking the photograph and Miss Anne Caisley for preparing Fig. 1.

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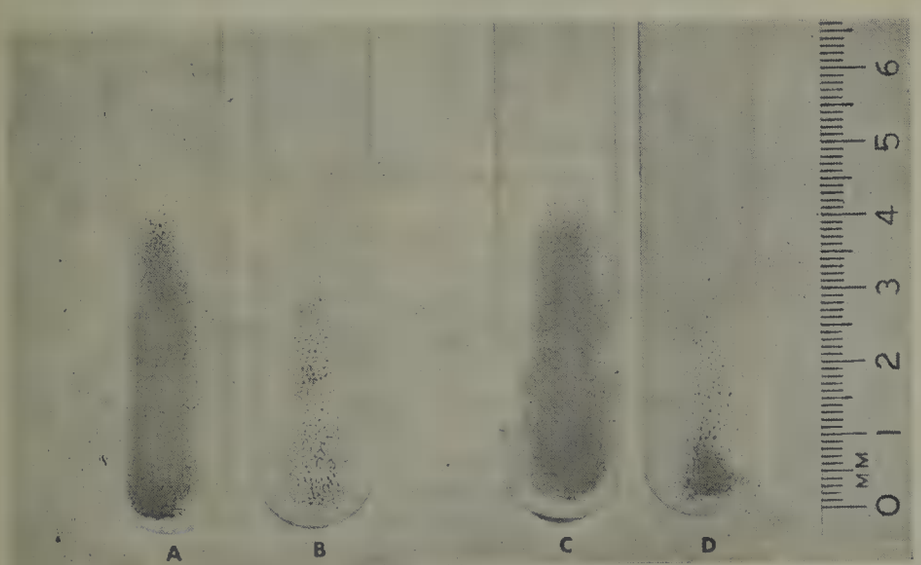

Fig. 2. *A*, *B*, Culture 7 days after seeding, stained with Mallory's stain: *A*, with raised cover-glass; *B*, without cover-glass. *C*, *D*, Culture 10 days after seeding, stained with haematoxylin and eosin: *C*, with raised cover-glass; *D*, without cover-glass

MICROBIOLOGY

Occurrence of Dimorphic Forms of *Mycobacterium phlei*

During the course of an investigation concerning the lipid fractions of *Mycobacterium phlei* it was found that liquid cultures which had been incubated for a period of 1 week at 37° showed the formation of a dry, rough pellicle. However, after incubation for 5 weeks only a sparse pellicle remained and the rough pellicle observed at 1 and 2 weeks had fallen to the bottom of the pan, forming a thick, mucoidal deposit. This deposit was unlike the granular deposit obtained in young cultures of mycobacteria and it was therefore decided to investigate the morphology of the organisms in these cultures. Using the method described in this communication, three morphological phases of growth from *M. phlei* were consistently obtained, which formed part of a definite cycle of growth.

Non-acid-fast stages in the life-cycle of different members of the Actinomycetaceae and Mycobacteriaceae have frequently been observed[1]. Rich[2] noted that considerable differences in the morphology of individual organisms could be found and this depended on the age of the culture, the character of the medium and the conditions of growth. Csillag[3] has obtained various non-acid-fast organisms from *Mycobacterium tuberculosis* var. *hominis* and atypical mycobacteria. The typical acid-fast bacilli were termed 'Form 1' mycobacteria and five different non-acid-fast types of organ-

ism were grouped together under the heading 'Form 2' mycobacteria. Csillag[4] found that a coccal form could be obtained from the 'Form 2' mycobacteria and regarded these as conforming with the definition of mycococci, which were originally described by Krassilnikov[5]. The genus *Mycococcus* is defined in *Bergey's Manual*[6] as consisting of cells of variable size and shape, generally spherical, occurring singly, in short chains or in clumps. Multiplication is by fission, constriction or bud formation. The cells are non-acid-fast, Gram-positive, aerobic and grow well on ordinary culture media.

The media used were: (*a*) 1 per cent glucose meat extract broth; (*b*) meat extract agar; this was prepared by mixing 1 lb. fat-free minced horse heart, 10 g peptone, 5 g sodium chloride and 1 l. tap-water, and heating for 30 min at 65°. The following day the mixture was heated for 2 h at 100°, filtered through coarse paper and the *p*H adjusted to 8·5. The broth was steamed for 45 min and the phosphate deposits filtered off on fine filter paper. Difco agar (14 g) was added and the mixture steamed for 30 min, filtered and sterilized at 15 lb. for 20 min; (*c*) a modified Sauton medium[7,8]: 5 lb. potatoes were left overnight in 4 per cent $KMnO_4$ solution, washed in distilled water, peeled and chipped. Distilled water (5 l.) and glycerine (1 l.) were added to the chipped potatoes and the mixture was left to infuse at 60° overnight. The potato extract was filtered off through gauze. The following ingredients were added to the extract: 48 g sodium glutamate, 3 g K_2HPO_4, 0·3 g brown ammonium iron citrate, 12 g citric acid, 3 g magnesium sulphate. The total volume was adjusted to 12 l. and the medium was steamed for 30 min. After filtering off any deposits the medium was distributed in 500-ml. amounts in 2-l. Caroll flasks (J. Jobling and Co., Ltd.) and autoclaved at 10 lb. for 10 min.

Cultivation of the organisms was as follows: A starter culture was prepared by inoculating a flask of 1 per cent glucose broth with *M. phlei* and this was incubated for 3–4 days at 37°. Part of the pellicle from this starter culture was transferred on a large loop to the surface of the Sauton medium. The cultures were incubated for six weeks at 37°.

Smears of the deposit in the Caroll flask were stained by the method of Gram and the Ziehl Neelsen method[9]. Examination of the stained preparations showed that 25 per cent of the cells were acid-fast and the remaining 75 per cent were non-acid-fast (Fig. 1), and these showed two different morphological types. The first of these was a Gram-positive rod with square ends; the rods sometimes appeared curved or bent double. The second type was a poorly stained Gram-negative organism containing a large clear ovoid inclusion.

Meat extract agar plates, previously incubated for 48 h to check sterility, were inoculated with the deposit from the 6-week-old cultures and incubated at 37°.

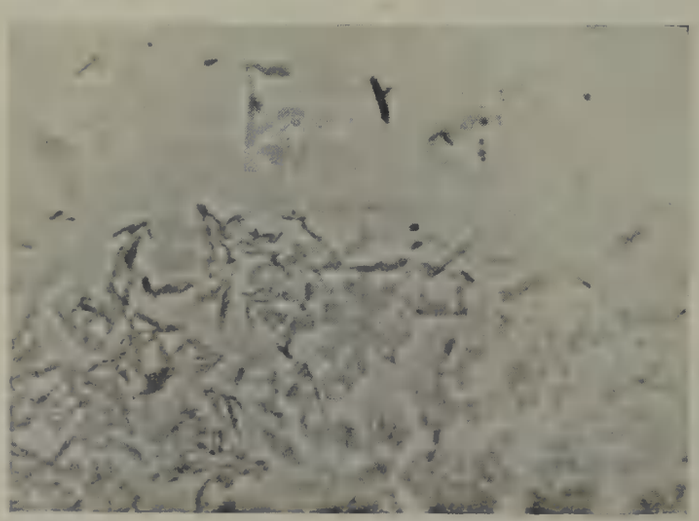

Fig. 1. Cellular morphology of a six-week-old culture of *M. phlei* (× *c.* 750)

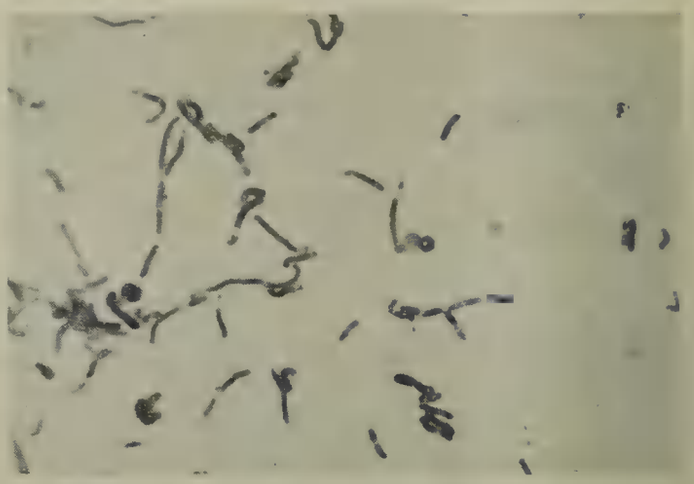

Fig. 2. Gram stain of Phase 1 of *M. phlei* (× *c.* 750)

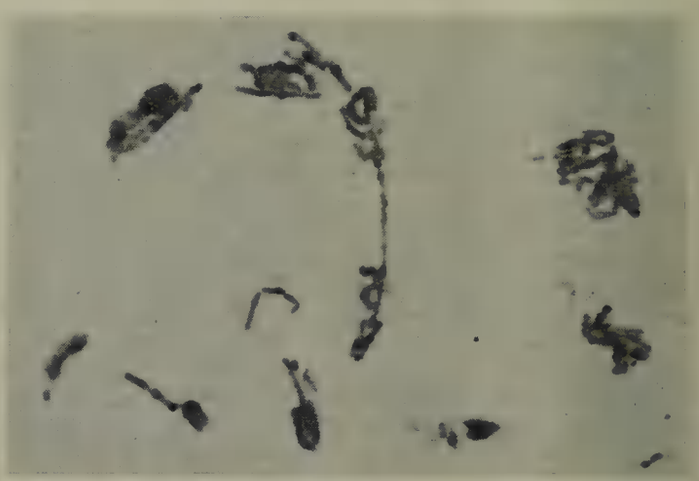

Fig. 3. Sudan black stain of Phase 1 of *M. phlei* (× *c.* 750)

After 36 h large flat colonies of acid-fast *M. phlei* were present; but after longer incubation small regular colonies, 0·4–0·6 mm in diameter, and larger irregular colonies, 2–3 mm in diameter, were found. From both types of colony sub-cultures were grown on meat extract agar. However, it was found that when these organisms had been sub-cultured several times the morphology, which had been checked at each stage, was obviously changing. Consequently, a further set of meat extract agar plates was inoculated with the rod-form, incubated for three days at 37° and then left at room temperature for one month. It was found that the colonies on these plates, which were 3–6 mm in diameter, contained coccal forms of variable size, arranged singly, in pairs or in clusters.

The morphology and staining reactions of the organisms isolated may be summarized as follows:

Phase 1. Large, non-acid-fast, motile, Gram-variable rods with a thick cell wall and a thin capsule. Each cell contained two or more large inclusions which were quite distinct and stained positively with Sudan black[9] (Figs. 2 and 3).

Phase 2. Small, Gram-negative, motile, pleomorphic organisms containing a large inclusion which almost filled the cell. These inclusions were sometimes acid-fast but only gave a very weak spore-stain reaction. After delipidation of the smear with alcohol–ether (1 : 1) for 2 min these ovoid inclusions were strongly stained by a spore-stain method[9]. It is possible that these reactions may be related to the acid-fastness (Figs. 4 and 5).

Phase 3. Gram-positive, capsulated, non-motile cocci of variable size, lying singly, in short chains or small clusters. These organisms contained no large inclusions and were non-flagellated (Fig. 6).

These phases 1 and 2 of growth from *M. phlei* are identical to two phases of growth included under the term 'Form 2' mycobacteria by Csillag[3]. Phase 3 from *M. phlei*

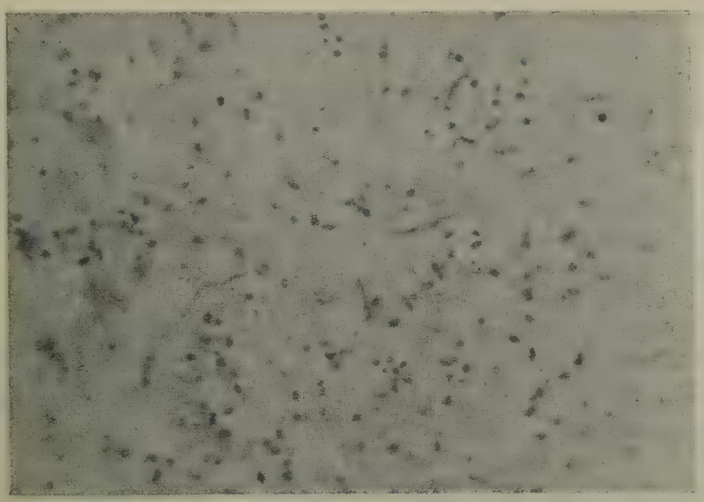

Fig. 4. Spore stain of Phase 2 of *M. phlei* after delipidation of the smear (× *c.* 750)

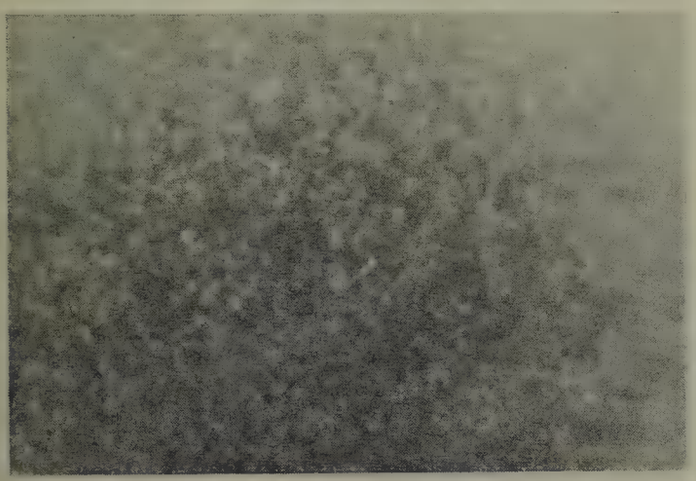

Fig. 5. Gram stain of Phase 2 of *M. phlei* showing the unstained ovoid inclusions (× *c.* 750)

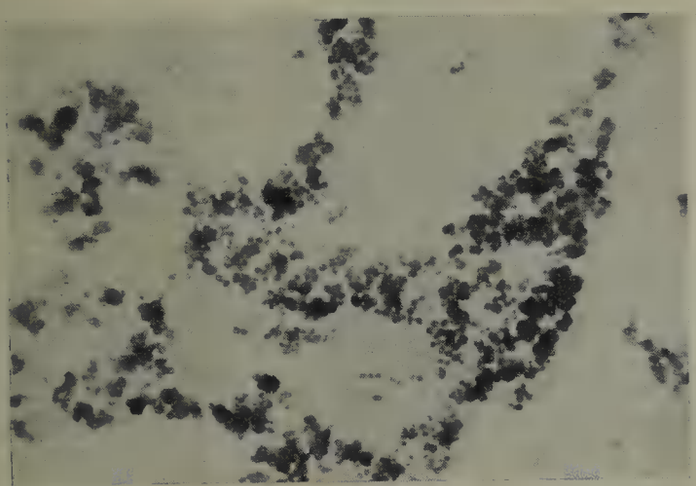

Fig. 6. Gram stain of Phase 3 of *M. phlei* (× *c.* 750)

corresponds to the 'mycococcal stage' isolated by Csillag[4]. Since it is possible to obtain the same phases of growth, the method described here has some advantages over previously reported procedures. The phases 1, 2 and 3 from *M. phlei* were obtained within 7 weeks without aeration of the cultures, whereas the 'Form 2' and 'mycococcal' stages were obtained after 14–52 weeks incubation with intermittent aeration[3,4].

The existence of a growth-cycle in mycobacteria has been suggested by various workers[10] although the precise nature of this cycle has never been determined. McClung[11] has described the cyclic nature of growth and the morphological changes which occur in the different species of the genus *Nocardia*. It is well known that a close relationship exists between the two genera *Nocardia* and *Mycobacterium*[12,13]. Consequently, if such a growth-cycle could be found in all species of mycobacteria there would be additional evidence to suggest that *Nocardia* and *Mycobacterium* should be included in the same family.

Further biochemical and serological experiments are being carried out with these phases of growth isolated from *M. phlei* in order to determine the nature of this growth cycle.

I thank Miss Kate McLean for her technical assistance in this work.

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VIROLOGY

Propagation and Properties of Hepatitis Virus

Testing a newly developed chemical reaction, we found the virus of hepatitis to consist of pure deoxyribonucleic acid without proteins, contrary to other known forms. This was investigated and confirmed as follows: by treatment with alcohol the virus can be precipitated like the nucleic acids and can be dissolved afterwards without loss of activity. With other virus such treatment led to total inactivation[1,2]. This property of the hepatitis virus enables one to prepare highly concentrated and pure samples, which do not contain proteins from tissues or other virus.

The hepatitis virus itself cannot permeate into the cell, the protein hull of virus being, as is well known, responsible for the affinity to the host cells. However, if the virus is brought into the cell by special treatment, then propagation and formation of plaques occur as with other viruses. The fact that, in such cases, the virus can be propagated in many tissue cultures confirms its character as a pure nucleic acid[3].

Enzymes that cleave nucleic acids degrade the virus, but trypsin, papain, Na-deoxycholate and ether are without any effect.

In vitro, the virus can be fixed by actinomycin like other deoxyribonucleic acids.

The following factors also indicate that the nucleic acid of the hepatitis virus is deoxyribonucleic acid (DNA): (1) Total inactivation by DNase, but not RNase; (2) 5-iodo-2′-deoxyuridine yielding an inhibition of propagation *in vivo*, while substances influencing the metabolism of RNA, such as 8-azaguanine, 2-thiouracil and 5-fluorouracil, have no influence; (3) actinomycin inactivates the virus *in vivo* as well as *in vitro*; it is known that actinomycin blocks DNA, but not RNA, *in vitro*; (4) estimation by the method of Schmidt and Thannhauser[5] confirms the DNA character of the virus.

The hepatitis virus can be propagated on the chicken embryo by the following technique: (1) Treating the chorio-allantoic membrane with hyaluronidase just before inoculation; this loosens the tissue and thereafter the virus can passively permeate into the tissue; (2) the

cytotoxic effect, especially from sera, can be removed by dilution, otherwise it prohibits multiplication of the virus; (3) the effect of DNA in cleaving enzymes such as DNase I and DNase of tissue origin will be greatly reduced by adding 0·01 moles Na-citrate and shifting the *p*H up to 8·5.

In tissue cultures the cells were washed before inoculation with 1 M NaCl, *p*H 8·2. The inoculation fluid contained hyaluronidase. Further propagation for many passages in tissue cultures is possible only if the *p*H of the medium does not go below 7, since *p*H 6·5 strongly decreases the active virus particles. The optimum *p*H for preservation of the hepatitis virus is between 8·5 and 9·5.

Plaque formation on the chorio-allantoic membrane appeared on the third day, and in liver and spleen on the fourth day after inoculation, the foci being similar to those of other virus, both morphologically and histologically[6]. In tissue cultures the beginning of the cytopathogenic effect was seen on days 3–4 after inoculation.

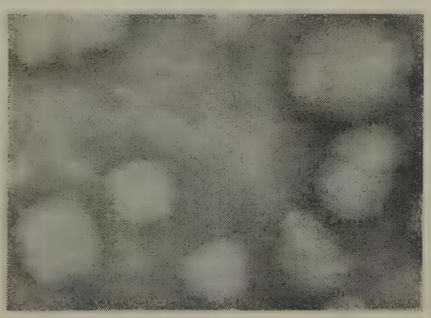

Fig. 1. Human hepatitis virus stained with uranyl acetate (× 100,000)

Electron microscopy shows the virus to have a polyhedral shape (Fig. 1). Negative staining with 1 per cent uranyl acetate makes it possible to measure the diameter, which is between 40 and 150 mμ. To determine whether the smaller particles are parts of larger ones, or are the virus itself, we separated the particles by ultracentrifugation. These were then tested for infectivity and diameters measured. The results showed that the smallest particles represent the virus itself.

The virus was propagated from 144 cases of acute and chronic hepatitis epidemica, from serum hepatitis and from a series of normal blood donors. The virus was found in sewage as well as in blood and in faeces. All 144 virus stems tested were identical in electron micrographs and by immunological tests.

For preparation of immune serum from rabbits, the injection of the pure virus suspension with Freund's adjuvant is recommended together with simultaneous application of anabolic steroids. With immune sera prepared in this way 144 stems were tested by neutralization and haemagglutination with erythrocytes of guinea-pig, and a complement fixation test. As has been stated, they all gave the same reaction.

Neutralizing and haemagglutination-inhibiting antibodies were found in patients after complete healing, while complement-fixing antibody was present as well as the virus only in chronically active cases. An efficient disinfectant is 'Havisol' (Schulke and Mayr G.m.b.H., Hamburg), based on phenol. The 6 per cent solution inactivates massive, protein-poor virus suspensions in 2 min. With more proteins present the virus was inactivated by 'Havisol' and 'Parmetol' in 2 per cent solution after 15 min.

As expected, the virus was much more resistant to heat than other viruses, and heating to 75° C for 1 h had no effect at all. Heating above 170° C was necessary to inactivate pure virus suspension with 10 P.F.U., within 1 h. The same suspension was inactivated in the autoclave after 30 min at 2 atmospheres at 134° C. With protein and cations, present heating for 1 h at 195° C, or at two atmospheres, was required to kill the virus. The addition of 2 per cent sodium carbonate led to the total loss of activity within 20 min at 100° C.

The hepatitis virus was more resistant to ultra-violet radiation. Irradiation of 0·5 ml. solutions in Petri dishes at a distance of 10 cm, using a mercury vapour lamp giving 2540 Å emission, produced an area of inactivation of 8·5 cm diameter after 6 min.

Since it was not possible to inoculate man with the virus, we performed the classical experiment of Neefe *et al.*[7] on human volunteers, as with the propagated virus in chicken embryo. The results were exactly as described by these authors.

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'Ring-forms' of Reovirus Particles

Several investigations utilizing electron microscopy have shown that during reovirus synthesis, progeny virus consisted of particles containing nucleic acid surrounded by protein coats (capsids), and others which were devoid of a central core[1–4]. Negative staining techniques[5] have afforded a means of following the course of events in infected cells.

The *L*-48 line of mouse fibroblasts was received from Dr. C. P. Stanners[6] and maintained in this laboratory in monolayer bottles in Eagle's medium with twice the usual concentration of amino-acids and 10 per cent calf serum. Cells were transferred every third day.

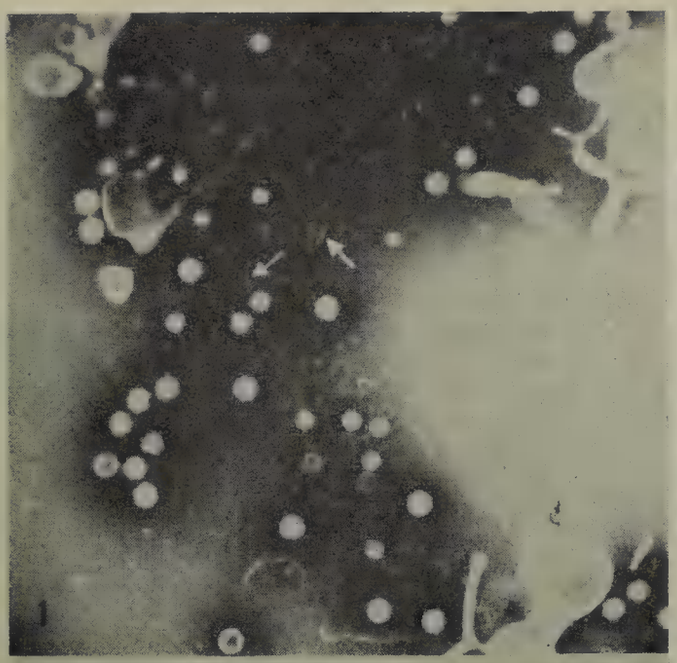
A type 2 strain of reovirus (988) (ref. 7) was prepared and concentrated according to methods previously described[8].

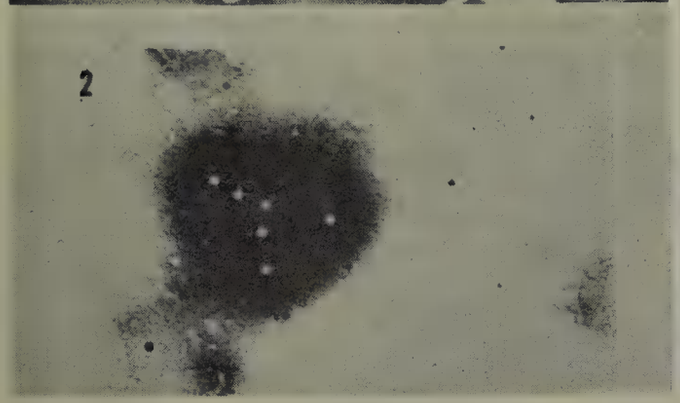
Sixteen-ounce monolayers of *L*-48 cells containing 1×10^7 cells were changed with 50 c.c. of pre-warmed medium (35° C) containing Eagle's as before, and 5 per cent foetal bovine serum which had been tested and found to be free of reovirus haemagglutinating inhibiting antibodies[7]. Each 10-oz. bottle was infected with 1×10^6 haemagglutinating units[9] of reovirus 988 in a 1-ml. volume. This ensured a multiplicity of virus to cell of 100–300 (ref. 10) allowing every cell in the culture to be infected at the time of virus inoculation.

The cultures were then incubated at 35° C for 1, 4, 12, 16, and 36 h. At each of these times supernatant medium was poured away, and the cells washed 10 times with 100 ml. of Earle's balanced salt solution (4° C). The remaining cells were then scraped from the glass with a rubber policeman, and allowed to swell in 3 ml. of a hypotonic medium (10^{-2} M *tris*, 10^{-2} KCl, 10^{-3} $MgCl_2$, *p*H 7·4) for 15 min (4° C). Cell membranes were then disrupted by 10 gentle strokes in a glass Tenbroeck homogenizer (4° C).

These lysates of virus-infected cells were examined on thinly spread films by electron microscopy. Crystalline bovine serum albumen was added to a final concentration of 0·286 mg/ml. to facilitate spreading. Five per cent phosphotungstic acid (PTA) neutralized (*p*H 7·0) with 1 M KOH was coated with a light film of talcum. One drop of cell lysate was then spread on to the surface. The films remained on the surface of the PTA solution for 5 min. They were lifted on to 'Formvar'-coated grids, and dried on filter paper. The grids were examined in an RCA *EMU 3E* electron microscope. Observations were made at initial magnifications of 5,000–18,000 diameters.

Virus particles were seen in preparations from infected cells as early as 1 h and as late as 36 h after infection. Complete virus was 69 mμ in diameter and contained a dense core of nucleic acid measuring 48 mμ. Enucleate





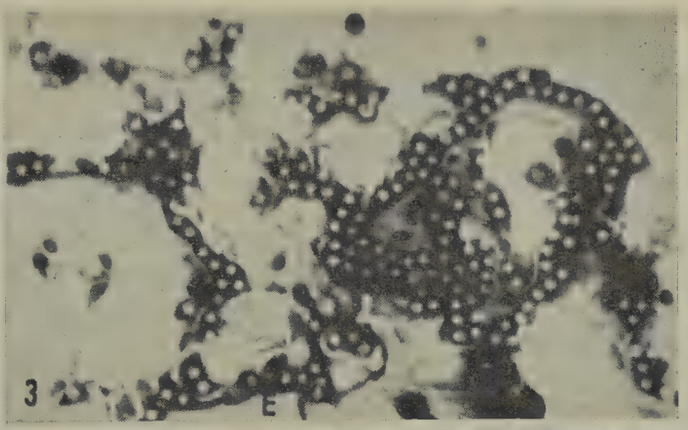


Fig. 1. Complete virus, enucleate capsids and a third type of particle, 'ring forms', are seen 12 h after infection. Complete virus and enucleate capsids are 69 mμ in diameter. The 'ring forms' (arrows) are enucleate and 48 mμ in diameter. (× *c.* 55,380)

Fig. 2. A group of nucleic acid cores in a preparation made 4 h after infection. (× *c.* 35,840)

Fig. 3. Free virus, cytoplasmic vesicles and canaliculi are seen in a preparation 16 h after infection. Capsomeres of the protein coat are discernible. There are enucleate particles and whole virus. (× *c.* 35,840)

Fig. 4. Sixteen h after infection, clusters of virus enclosed by a limiting membrane or sac are seen (lower right). (× 61,800)

particles with a protein coat of capsomeres (capsid) but without central cores were present in all preparations.

At 12 h or more following infection of *L*-cells by reovirus, several changes in virus progeny were observed. In addition to complete and coreless forms, a third type of particle was noted in some fields. These were empty particles with an outer limiting membrane. Capsomeres were not seen (Fig. 1). These 'ring forms' had a diameter of 48 mμ, equal to that of the central core of the complete particle. A fourth type of virus material of similar dimension was also observed. These were solid electron-dense polygonal particles (Fig. 2) which appear to be the nucleic acid or core material.

At 16 h after infection aggregations of virus particles enclosed within a limiting membrane or sac were seen (Figs. 3 and 4). Several points concerning reovirus became evident. The appearance of ring forms at 12 h after infection suggests new virus synthesis. The ring could well be protein. This suggests that reovirus protein consists of 2 morphological types, capsomere and 'ring'. The inner layer of the ring was noted by Vasquez and Tournier[3]. It had never been observed free of capsomere protein, and the time of appearance was not described. 'Ring forms', which have a diameter equal to that of the nucleic acid core, could serve as an outer covering of the nucleic acid or as an inner lining for capsomeres.

The observation of virus aggregates in a sac is reminiscent of an earlier observation by Horne and Nagington for poliovirus[11]. Such a cytoplasmic structure has recently been shown to be involved in the synthesis and assembly of poliovirus components[12].

This work was supported by grants 1 *T*1 *A*1 261–01 and *A*1–05721–01*A*1 from the U.S. National Institutes of Health, and *G*–24381 from the U.S. National Science Foundation.

Note added in proof. Findings similar to those reported here have appeared in two recent publications from another laboratory: Mayor, H. D., and Jordan, L. E., *Exp. Mol. Path.*, **4**, 40 (1965). Mayor, H. D., Jamison, R. M., Jordan, L. E., and Mitchell, M. Van, *J. Bact.*, **89**, 1548 (1965).

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GENETICS

'Complement Fractionation' in a Natural Hybrid between *Rubus procerus* Muell. and *R. laciniatus* Willd.

Rubus procerus Muell. and *R. laciniatus* Willd. occur sympatrically over a wide range of area in the coastal Pacific north-west of the United States. Natural hybridization between these two pseudogamous apomictic species has been shown to take place by Bammi and Olmo[1]. One such partially sterile natural hybrid (6412*A*), collected from Riverton, Oregon, proved very interesting cytologically. The present report deals with the peculiar cytological behaviour of this hybrid clone.

The chromosome analyses in this study were made from temporary squash preparations according to the alcoholic-carmine technique described by Snow[2].

All clones of *R. procerus*, *R. laciniatus* and the hybrid clone were tetraploids with 28 chromosomes in their somatic cells. Stages prior to diakinesis or metaphase I were not suitable for analyses.

Both *R. procerus* and *R. laciniatus* behave as segmental allopolyploids. Usually not more than 1–2 quadrivalents are formed at microsporogenesis. The hybrid clone (6412*A*) showed a peculiar kind of abnormality. In about 60 per cent of the microsporocytes two chromosomal plates were present, instead of the expected one, at metaphase I. These two plates behaved as independent units during the rest of the meiosis. Various other abnormalities like non-disjunction, lagging of certain chromosomes at first and second division of meiosis, separate or in conjunction with the above described abnormality, were also observed. About 1·5 per cent of the microsporocytes in *R. laciniatus* also showed more than one group of chromosome at metaphase I. The net result of the phenomenon of multiple plates at metaphase I and the behaviour of these groups of chromosomes to operate independently of each other in subsequent stages results in the production of an excess of sporads at telophase II. Table 1 shows that at telophase II, this plant had only 29–30 per cent of the cells with four sporads. This is in contrast to the clones of the parents and the hybrids, where cells with four sporads were about 97–99 per cent of the total. Almost all the rest of the cells had excess sporads.

Table 1. Frequency Distribution of Cells at Telophase II with regard to Number of Sporads per Cell in the Suspected Hybrid Clone 6412*A*

Total cells studied		Cells with sporads numbering								
	4+ Micronuclei	1	2	3	4	5	6	7	8	9+
273	No. 18	1	3	8	80	42	65	12	42	2
	Percentage 6·59	0·37	1·10	2·93	29·30	15·38	23·81	4·40	15·38	0·74

Thompson[3] introduced the term 'complement fractionation' for the general phenomenon wherein "the chromosome complement is sub-divided into independently operating groups within a cell". Thompson[3], in working with the cultivated variety 'Boysen' of the genus *Rubus*, found a similar situation of sub-division of the chromosome complement into independently operating groups within meiotic cells. In her interpretation there were no definite implications as to the genetic constitution of the end-product. However, the present study goes a step further in proposing that the two sub-groups of chromosomes at metaphase I in fact belong to different genomes, namely *R. procerus* and *R. laciniatus*. This interpretation is based on the following observations.

(1) Sub-division does not seem to be a completely random process. The number of bivalents in each group at metaphase I tends to be 7 (14 being the gametic number for both *R. procerus* and *R. laciniatus*).

(2) Rarely, more than 2 sub-groups were observed at metaphase I.

(3) Each sub-group seems to have a separate spindle. This is supported by the following observations:

(*a*) Lack of synchronization of 2 groups in some cells, and (*b*) their separation in different planes in a 3-dimensional cell.

(4) Counts made at anaphase II show that chromosomes number in each group tends to be 7.

Since the frequency of this phenomenon is different in different clones, it is assumed to be genotypically controlled.

No definite conclusion as to the time or method of origin can be drawn from this work. Some indication of sub-grouping of chromosomes in somatic cells was also seen. It is probable that actual separation of genomes had taken place in the premeiotic cells.

The presence of 'complement fractionation' in the cells of an organism has many implications. It may be an indication of its obvious or concealed hybridity. It is quite conceivable that one of the resulting gametes may be functional and may take part in fertilization. This could result in the formation of unique segregants which would be expected to be rare or impossible in the normal reproductive pattern. As pointed out by Thompson[3], the separation of single genomes which form functional gametes can have value in the discovery of ancestral species of polyploids. There, of course, then exists the fascinating possibility that the genome of an ancient diploid trapped inside the constitution of a polyploid (in this case, an apomictic one) might also be released. This phenomenon might also provide a method by which the polyploid level of a species may go down.

Examples of 'complement fractionation' have so far been reported in cultivated plants of hybrid origin, for example by Thompson[3] and in tomato by Gottschalk[4], and in *Rubus* by Hull and Britton[5], that were previously subjected to colchicine treatment. This is probably the first instance where a naturally occurring plant shows this phenomenon.

I thank Prof. H. P. Olmo for advice.

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SOIL SCIENCE

Thermistor Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils

In any investigation involving the movement of moisture through soils, the measurement of the free energy or total potential of the soil moisture still remains one of the chief problems. The basic requirement of any satisfactory technique for such measurement is that it should depend on some property which is uniquely related to the free energy of the soil moisture and is in no way affected by the soil type. One such property is the relative vapour pressure or humidity in equilibrium with the soil moisture as given by equation (1).

$$h = \frac{RT}{Mg} \log_e \frac{p}{p_0} \qquad (1)$$

where h = free energy (cm water); R = universal gas constant; T = abs. temp.; M = molecular wt. of water; $g = 981$ cm/sec^2; $\frac{p}{p_0}$ = relative humidity.

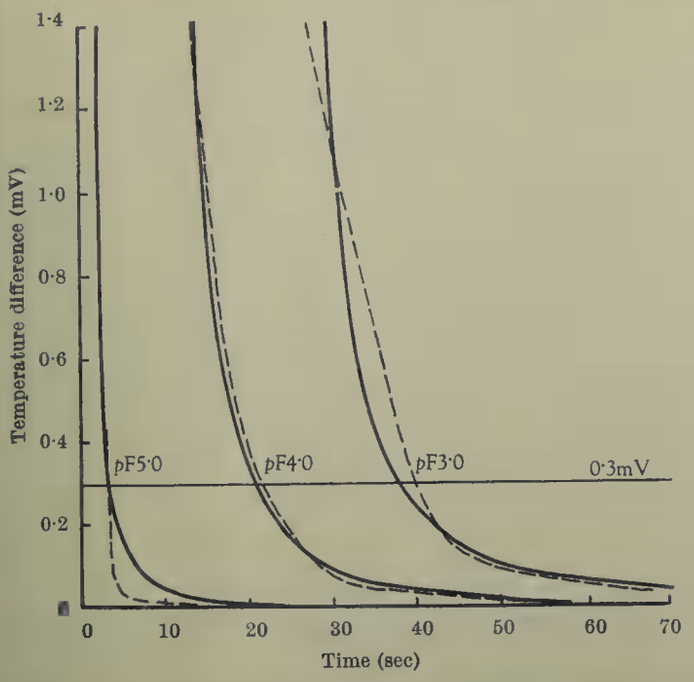

Fig. 1. Comparison of theoretical (solid lines) and measured curves (broken lines)

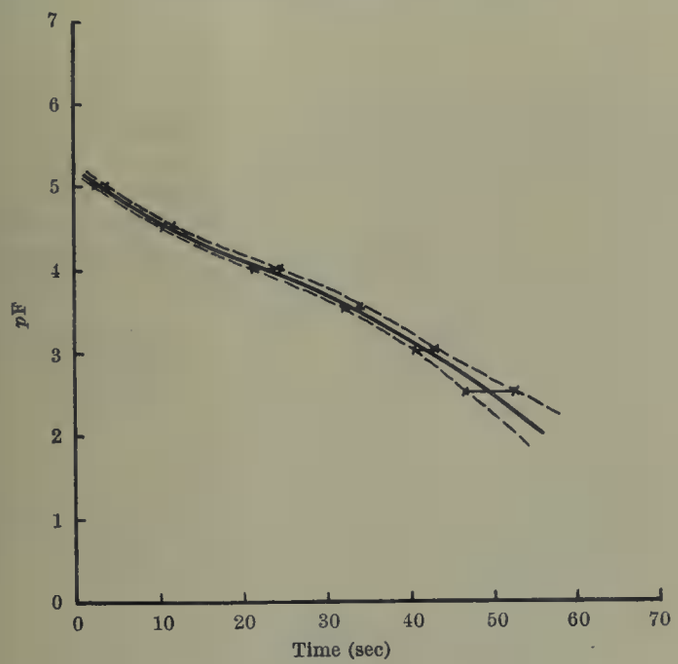

Fig. 2. Probe *P*; time to reach 0·3 mV for 16-h reading

The amount of capillary condensation in a porous medium from some fixed initial condition is closely related to the ambient humidity. The porous medium chosen for study was the micro-porous glass coating on a thermistor bead. It has been shown[1,2] that glass surfaces may have effective surface areas many hundred times greater than the nominal area of the surface. The initial condition was established by heating the thermistors with their normal operating current in the room atmosphere at approximately 60 per cent humidity for 1 h. The capillary moisture which afterwards condensed on the glass surface could then be measured by the heat energy required to vaporize this moisture or more simply the time delay in the temperature rise as current was passed through the thermistor.

The original experimental work was carried out using a two-thermistor probe sealed into a sample tube. One thermistor, enclosed in an air-tight compartment brought to approximately 0 per cent humidity with silica gel, was used as the standard in a Wheatstone bridge circuit with a 1·35-V d.c. supply from a mercury cell, while the second thermistor was open to the sample atmosphere. The thermistors were 'zeroed' to their 'final' condition by passing the current for 1 h. After allowing a period of 16 h for capillary condensation to take place, the current was again switched on. Typical readings are shown in Fig. 1 for Stantel '*P*23' thermistors and for standard sodium chloride solutions supported on filter paper.

Although the standard thermistor compensated for small changes in mean temperature, differences in temperature between the thermistors and the sample could cause serious errors. These errors were minimized by carrying out the experimental work in a constant temperature room ($\pm 0{\cdot}1^\circ$ C) with the sample tube and probe placed in an 18-in. cube of polystyrene insulating foam.

Fig. 1 also shows theoretical curves of the temperature difference between a '*P*23' thermistor in dry air (that is, the standard) and the second '*P*23' thermistor also in dry air, but after time delays corresponding to the experimental curves. These curves were calculated[3] assuming the heat is produced in a sphere of perfect conductor surrounded by an infinite region.

In most of the later work, the whole temperature curve was not recorded, and only the time to reach a temperature difference corresponding to a bridge output of 300 μV was measured on a stop-watch. This gave a satisfactory curve between Schofield's *p*F and the reading in seconds between *p*F 2·5 and 5·0 as shown in Fig. 2. During the duration of the test, a period of about 2 months, no surface contamination of the porous surface was apparent. Similar curves were also obtained for other types of thermistors and for other capillary condensation times. Times less than 1 h still gave satisfactory calibration curves for more rapid measurements in the range *p*F 2·5 to 5·0, but with a lower sensitivity.

In the present work a new simple two-thermistor probe is being used with the standard coated with water repellant. Although this work is not complete, similar curves are being obtained to that shown in Fig. 2, using equipment that can be purchased for a few pounds. This technique shows considerable promise in the measurement of free energy of soil moisture in the range *p*F 2·5–5·0.

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[1] Adam, N. K., *The Physics and Chemistry of Surfaces* (Oxford, London, 1941).
[2] Imelik, B., *Proc. Tenth Symp. Colston Res. Soc.*, Bristol (1958).
[3] Carslaw, H. S., and Jaeger, J. C., *Conduction of Heat in Solids* (Oxford, London, 1959).

Photographic Reproduction of Soil Fabric Patterns

An important prerequisite for a satisfactory fabric analysis of any complex, multi-component fabric pattern is the requirement that the component individuals can be relatively easily recognized as to their nature and their geometrical relationships towards adjacent individuals. In rocks, for example, the various mineral individuals can, under average conditions, be recognized readily and clearly distinguished from adjacent crystal individuals. As a result, rock fabric analysis can usually be carried out directly on the image provided by the microscope.

The microscopical image of soil fabric patterns is, as a rule, very complex. Thus, particularly in spatial distribution analysis[1], the nature of the individuals of the various components may be rather difficult to establish, or boundaries between components may appear hard to locate. For this reason, even elementary soil fabric analysis tends to develop into an extremely slow, tiresome and costly process, particularly if areas larger than the field of the microscope or even whole thin sections are involved.

The obvious solution to this problem is to replace the original microscope image of the pattern by a simplified

Table 1. Photographic Effects of the Various Steps in the Procedure

Step No.	Photographic product	Type of light	Appearance of 'primary' fabric elements		
			Skeleton grains	Clay matrix	Pores
Step 1 *A*	Negative	Ordinary parallel light	Black	White*	Black
B	Positive transparency	Ordinary light, parallel beam or diffuse	White	Black	White
Step 2	Negative	Plane polarized light, rotating crossed Nicols	Black	White	White
Step 3	Combination of positive transparency (1*B*) and negative (2)	Ordinary parallel light	See step 4	Black	White
Step 4	Negative	Ordinary parallel light under an angle of approx. 70°–80°	White areas surrounded by fine, black lines	White	Black
Step 5	Print or enlargement of negative (4)	Ordinary light, parallel beam or diffuse	Black areas surrounded by fine, white lines	Black	White

* Depending on the colour of the clay material in the thin-section. If sufficiently reddish, the result can be obtained by using the appropriate type of photographic emulsion. If the colour of the clay material is insufficiently reddish, a suitable preferential staining of the clay can be considered.

graphical model which contains all the required information but no unnecessary details. The ideal graphical model therefore should possess the following major properties: it should show all relevant constituents in their true size and shape ratios, and in their mutual spatial relationships; it should also show all the individuals of any one constituent in the same way (for example, skeleton grains in black, clay matrix in grey, pores in white, etc.). Simple drawing or tracing of the microscope image seems attractive, but is, particularly with the usually complex soil fabric patterns, extraordinarily time-consuming and often insufficiently accurate. Ordinary photomicrography, also in polarized light between crossed Nicol prisms, is accurate indeed, but still has the main disadvantage of complexity of the original microscope image, and in addition shows the loss of a number of diagnostic characteristics (for example, extinction angle between crossed Nicol prisms, birefringence, etc.). In many instances these characteristics must be determined later in the original microscope image, leading again to an excessive loss of time.

Over some period of time we have tested several combinations of photomicrographic and drawing techniques. Some of these have been applied for a time, but have been rejected, mainly because they were too time-consuming. We have finally adopted a method which virtually satisfies the two conditions of the ideal model. It is a purely photographic technique which does not demand an excessive amount of time spent on routine work, but rather calls for appreciable photographic experience and skill, as well as for very accurate working methods.

The steps in the actual operation can briefly be described as follows:

1. (*A*) The area of the soil thin section to be investigated is photographed at the desired magnification and in ordinary transmitted light. (*B*) From the negative obtained a positive transparency is made by contact printing.

2. The same area of the thin section is then photographed again without altering the position of camera, microscope or thin section. The exposure is now made, however, in plane polarized light between crossed polarizing filters or Nicol prisms. During exposure the crossed polarizing filters or prisms are simultaneously rotated through an angle of at least 180°. The result of this exposure is a negative, which is directly used in the next step.

3. The positive transparency of step 1*B* and the negative of step 2 are now put together with the sensitized sides of both film-sheets on the outside and the corresponding parts of the images fitting very accurately. The fitting together of the corresponding parts of the images is the most critical part of the whole procedure, and must be carried out with the utmost care to ensure success of the whole operation. Once the fit of the two film-sheets is perfect, they should be joined semi-permanently or permanently.

4. From the joined set of the positive transparency and the negative, a new negative is now produced in the following manner. The combined two film-sheets, with the third, new film-sheet directly underneath, are exposed to a beam of parallel light (for example, from a photographic enlarger or a microscope lamp) in such a way that the plane of the three film-sheets makes an angle of about 70°–80° with the axis of the light beam. While exposing, the stack of three film-sheets, maintaining the previously mentioned angle of 70°–80°, is rotated through an angle of at least 360° around the axis of the light beam.

5. The new negative produced in step 4 can now be printed or enlarged in the ordinary way.

The final result of the whole operation is demonstrated in Fig. 1, which shows an area of a thin section of an arid red earth from Alice Springs, N.T., Australia. Nearly all skeleton (quartz) grains are shown as black areas surrounded by a fine white line. The very few quartz grains which are shown as white or whitish areas in the figure are those which in the original thin section were cut perpendicular or nearly perpendicular to their optical axis. The majority of these reveal themselves in the photograph immediately by their size and shape. In only very few instances will it be necessary to return to the original thin section for additional information. In the same figure the clay matrix is shown in solid black, and the pores in solid white.

The results and effects of the various steps are described in Table 1.

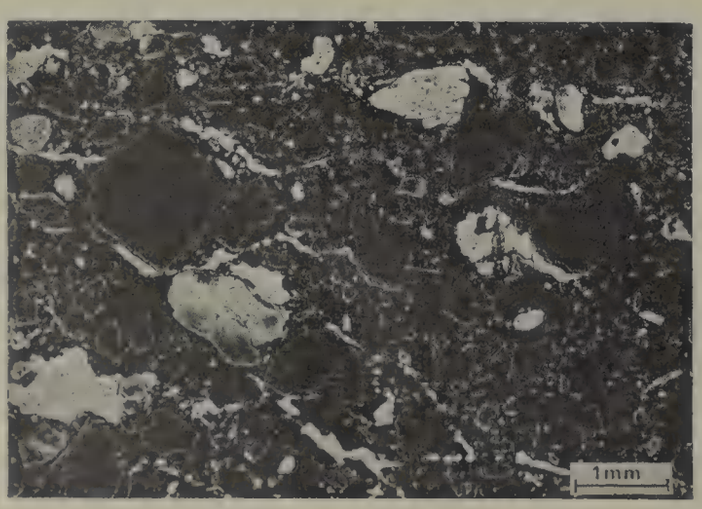

Fig. 1. Photographic reproduction of the fabric pattern of an arid red earth, Alice Springs, N.T., Australia. (Depth of the sample below the surface: 5 ft. 6 in.)

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[1]Lafeber, D., *Proc. Fourth Austral. N.Z. Conf. Soil Mech. Found. Eng.*, 1963: 185 (1964).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 8

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. R. A. Horsley: "Creep Properties of Thermoplastics".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. G. P. C. Chambers: "The Colloidal and Surface Properties of Clay Minerals".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Hatfield College of Technology, Hatfield), at 6 p.m.—Discussion on "Is Laboratory Work Really Necessary?" opened by Dr. K. R. Sturley.

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fuel Element Behaviour in Gas-Cooled Reactors".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. A. G. Page: "Some Manufacturing and Processing Techniques in the Electronics Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Lord Rennell of Rodd: "Heinrich Barth and the Opening Up of Central Africa".

Tuesday, November 9

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Homo Viator and Homo Sapiens".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 2.30 p.m.—Mr. P. R. Barnard and Mr. R. Paul Johnson: "The Ultimate Strength of Composite Beams"; Mr. R. Paul Johnson, Mr. C. H. Finlinson and Mr. J. Heyman: "The Plastic Behaviour of Continuous Beams" and "A Plastic Composite Design".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. E. Hesmondhaigh: "The Design of the A.C. Servomotor".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. L. R. Mernagh: "Tyres 1965". 7 p.m.—Dr. A. Schallamach: "Tyre Wear is Complex".

ROYAL INSTITUTION (at 21 Albermarle Street, London, W.1), at 5.30 p.m. —Sir John Baker, O.B.E., F.R.S.: "The Excitement of Engineering Design" (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on November 10, 16 and 17).

ROYAL INSTITUTION OF NAVAL ARCHITECTS (joint meeting with the Institute of Marine Engineers, in the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. A. F. Willens and Mr. D. R. Murray Smith: "Fire Fighting and Fire Protection in Ships".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. D. Rogers: "The Portraiture of Molecules" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. III. Emergence of the Typhus Group of Fevers".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. A. R. Reece: "Principles of Soil-Vehicle Mechanics".

UNIVERSITY OF LONDON (in the Mechanical Engineering Building, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Waves". (Third of four lectures on "Magneto-Fluid Dynamics".)*

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. J. Z. Young, F.R.S.: "The Memory in the Brain" (Distinguished Visitors' Address).

Tuesday, November 9—Thursday, November 11

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Convention on "Interaction Between Vehicle and Track".

Wednesday, November 10

POLAROGRAPHIC SOCIETY (at the National Physical Laboratory, Teddington, Middlesex), at 2.30 p.m.—Meeting on "E.S.R. and Polarography".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m. Meeting on "Automatic Analysis".

ENVIRONMENTAL GROUP (in the Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.— Discussion on "The Adequacy of our Current Measures of the Environment".

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6 p.m.— Prof. C. S. Barrett (University of Chicago): "X-ray Diffraction at Low Temperatures".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./ I.E.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. D. W. Hill: "Medical Electronics Round the World".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. G. M. E. Williams: "Control in Automatic Mechanical Production".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. Hudson: "Diversification in Packaging".

Wednesday, November 10—Thursday, November 11

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2)—Conference on "Power Applications of Controllable Semiconductor Devices".

Thursday, November 11

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. S. W. Kuffler (Harvard Medical School): "Physiological Properties of Vertebrate and Invertebrate Neuroglial Cells and the Movement of Substances Through the Nervous System" (Ferrier Lecture).

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Access to Airports" introduced by Mr. C. A. Harvey.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. IV. The Imphal Circus".*

CHEMICAL SOCIETY (in the Chemistry Lecture Theatre, King's College, Strand, London, W.C.2), at 6 p.m.—Dr. B. A. Thrush: "The Formation of Electronically Excited Molecules in Simple Gas Reactions" (Tilden Lecture).

UNIVERSITY OF LONDON (in the Mechanical Engineering Building, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Two Weird Boundary Layers". (Last of four lectures on "Magneto-Fluid Dynamics".)*

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. F. G. Dunkley: "Airless Spray Painting".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN AND THE OSLER CLUB (at 17 Bloomsbury Square, London, W.C.1), at 8 p.m.—Dr. F. Guerra and Prof. F. E. Camps: "Phantastica (Drugs, Dreams and Addictions)".

Friday, November 12

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m. —Symposium on "Yield and Quality in Potatoes".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Meeting on "The Flora Europaea Project". Speakers to include Prof. H. Merxmüller (Munich), Prof. D. A. Webb, Prof. D. H. Valentine, Prof. J. Dostál (Czechoslovakia) and Dr. S. M. Walters.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. S. Callender: "Iron Absorption".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. B. M. Weedy and Mr. A. M. Parker: "Method of Predicting the Thermal Loading of an Oil Circuit Breaker".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. M. J. Langeveld (Utrecht): "Educating a Whole World".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.— Dr. J. R. Napier: "Evolution of the Human Hand".

Saturday, November 13

INSTITUTE OF LINGUISTS (at the British Academy, Burlington Gardens, London, W.1), at 3 p.m.—Mr. Peter F. D. Tennant, C.M.G., O.B.E.: "Language, Communication and Misunderstanding" (Sixth Threlford Memorial Lecture).

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. B. A. L. Cranstone: "The British Museum Expedition to New Guinea".*

Monday, November 15

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10.30 a.m. and 2.30 p.m.—Colloquium on "Phase Measurement Throughout the Spectrum".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. D. E. Stevenson: "The Assessment of Possible Health Hazards Associated with the Use of Pesticides".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. V. Old and New Horizons".*

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. —Discussion on "The Engineer and the Law".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Colin Cherry: "The Nature of Human Communication". (First of three Cantor Lectures on "World Communication".)

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the College of Further Education, Hatfield Road, St. Albans), at 7.30 p.m.—Dr. J. S. Gourlay and Dr. C. K. Warren: "The Chemist's Contribution to the Building Industry of the Future".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT IN VETERINARY CLINICAL BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (November 10).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Newcastle upon Tyne, 2 (November 12).

LECTURER or ASSISTANT LECTURER (with considerable field experience of applied geology, preferably on the side of engineering) IN GEOLOGY AND MINERALOGY—The Secretary, The University, Aberdeen (November 13).

LECTURER (COMPUTER MANAGER) (graduate with experience of computer systems and administration); and ■ SENIOR LECTURER (with suitable experience in some aspect of computing science such as system programming) IN THE COMPUTING DEPARTMENT—The Secretary of University Court, The University, Glasgow (November 15).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leeds, 2 (November 15).

PROFESSOR IN MATHEMATICAL STATISTICS AT THE UNIVERSITY OF CAPE TOWN—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (November 15).

LECTURER in CONTROL or DYNAMICS IN THE DEPARTMENT OF ENGINEERING—The Registrar, The University, Leicester (November 17).

SENIOR LECTURER and a LECTURER IN THE NEW LABORATORY OF GENERAL PHYSIOLOGY—The Registrar, The University, Leicester (November 17).

HEAD OF THE DEPARTMENT OF BIOLOGY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (November 19).

LECTURER or ASSISTANT LECTURER IN MEDICINE—The Registrar, The University, Sheffield (November 20).

LECTURER or ASSISTANT LECTURER (preferably specialized in modern analysis or topology) IN PURE MATHEMATICS—The Registrar, The University Sheffield (November 20).

UNIVERSITY DEMONSTRATOR (with an honours degree and postgraduate teaching and/or research experience) IN AGRICULTURE—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (November 20).

LECTURER (preferably with an interest and competence in physiological and animal psychology) IN PSYCHOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 22).

LECTURER (qualified in chemistry, physics, or a branch of technology, and with industrial or postgraduate experience in a branch of materials science) IN MATERIALS AND MOLECULAR SCIENCE—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (November 22).

LECTURER/SENIOR LECTURER in BIOCHEMISTRY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 26).

TEACHING FELLOWS/DEMONSTRATORS (2) (preferably with an honours degree in rural science, agriculture or science with specialization in plant sciences) IN AGRONOMY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 26).

CHAIR OF ZOOLOGY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Kuala Lumpur, November 30).

HEAD (senior research worker, preferably holding veterinary or medical qualifications) OF THE PHYSIOLOGY AND BIOCHEMISTRY DEPARTMENT—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (November 30).

LECTURER (preferably with a veterinary qualification and interested in chemical pathology) IN THE DEPARTMENT OF PATHOLOGY—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (November 30).

PROFESSOR OF PHYSICAL CHEMISTRY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

SENIOR LECTURER and a LECTURER IN SOCIAL ANTHROPOLOGY IN THE DEPARTMENT OF AFRICAN STUDIES, University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

SUPERVISING GEOLOGIST (with a university degree with geology as a major subject, extensive experience in the examination and assessment of mineral deposits, together with administrative ability) IN THE GEOLOGICAL SURVEY BRANCH OF THE DEPARTMENT OF MINES—The Public Service Commissioner, 184 St. George's Terrace, Perth, Western Australia (December 3).

LECTURER or SENIOR LECTURER IN CHEMICAL PATHOLOGY at the University of Otago Medical School, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (New Zealand and London, December 15).

ANIMAL NUTRITIONIST (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture with post-graduate training or experience in biochemistry or agricultural biochemistry) with the East African Agriculture and Forestry Research Organisation, to investigate metabolism of tropical and temperate type cattle with a tropical environment—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. PC213/214/016.

CHAIR OF PHILOSOPHY—The Dean, Faculty of Arts and Science, McGill University, Montreal, Canada.

CHIEF TECHNICIAN (preferably with some knowledge of glassblowing and experience with instrumental analysis, for example, gas chromatography) to take charge of chemical laboratories and stores in the Chemical Engineering Department—The Establishment Officer, University College, Gower Street, London, W.C.1, quoting Ref. Chem. Eng /5.

LECTURER or ASSISTANT LECTURER (with a degree in physics or general science and/or an ophthalmic optical qualification) IN THE DEPARTMENT OF OPHTHALMIC OPTICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

MASTER (preferably graduate in mechanical sciences or engineering) to teach MATHEMATICS and some PHYSICS—The Headmaster, The School, Oundle, Peterborough.

RESEARCH ASSISTANT IN THE DEPARTMENT OF METALLURGY to study the behaviour of point defects in metals using electron microscopy and electrical resistivity techniques—The Registrar, University College of Swansea, Singleton Park, Swansea.

RESEARCH TECHNICIAN (preferably with some experience in growing plants under controlled conditions and of chemical analysis) to assist Prof. J. F. Sutcliffe in an investigation of the absorption and movement of mineral salts in intact plants—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The National Plan. (Cmnd. 2764.) Pp. xviii+474. (London: H.M. Stationery Office, 1965.) 30*s*. net. [219

Bacteria and the Biochemist. By Prof. S. Dagley. (An Inaugural Lecture delivered before the University of Leeds on 23 November, 1964.) Pp. ii+23. (Leeds: The University, 1965.) 2*s*. 6*d*. [110

Ministry of Agriculture, Fisheries and Food: National Agricultural Advisory Service. Experimental Husbandry Farms and Experimental Horticulture Stations—Sixth Progress Report. Pp. v+70+4 plates. (London: H.M. Stationery Office, 1965.) 6*s*. 6*d*. net. [110

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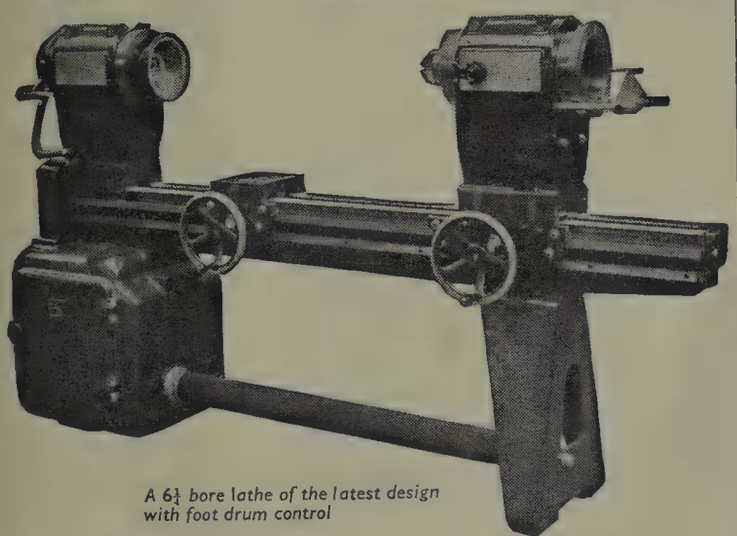

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(c) Senior Lectureship or Lectureship in Zoology. Special interest in fresh-water ecology or hydrobiology essential. Appointment to commence as soon as possible.

Salary scales (including expatriation allowances): Senior Lecturer, £2,100 to £2,660 per annum; Lecturer, £1,550 to £2,350 per annum (at present both under review). Appointments on permanent terms or contract terms carrying 25 per cent gratuity (15 per cent if in F.S.S.U.). Subsidized accommodation. Expatriate staff receive: education allowances; family passages; and biennial overseas leave.

Detailed applications (six copies), naming three referees by November 29, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom full details may be obtained.

THE POLYTECHNIC

309 REGENT STREET, W.1

DEPARTMENT OF MATHEMATICS AND PHYSICS

Applications are invited for the full-time posts of Senior Lecturer in Mathematics, Lecturer or Grade B Assistant Lecturer in Mathematics, Lecturer or Grade B Assistant Lecturer in Physics. The lecturing work involved is with undergraduates preparing for B.Sc. degrees in mathematics, physics or engineering or the general degree in mathematics and physics. Candidates should possess an honours degree. Research is encouraged. Salary will be in accordance with the Burnham Technical Report (at present under review) proposed scales being: Senior Lecturer, £2,185 to £2,425; Lecturer, £1,920 to £2,185; Assistant Grade "B," £1,100 to £1,770 with appropriate training and graduate additions.

A form of application, which should be returned not later than November 12, 1965, may be obtained on application to the undersigned.

J. E. RICHARDSON,
Director of Education.

BRITISH EGG MARKETING BOARD

SCIENTIFIC AND TECHNICAL DIVISION

CHIEF CHEMIST

Applications are invited for the above appointment which will shortly become vacant. For this senior position it is anticipated that the successful candidate will hold a degree with honours in one or other scientific disciplines—Agricultural Chemistry, Chemistry or Biochemistry. He will lead a small team of technical staff who are engaged on investigations affecting the production, quality control and use of eggs and egg products. Ideally he should be between 30 and 45 years of age and should have a keen interest in the application of basic scientific knowledge to the practical problems associated with the British poultry industry. It would be an asset, therefore, although not essential, if candidates have a knowledge or background in agriculture.

The working facilities are situated in the Board's Headquarters at Wingate House, Shaftesbury Avenue, and consist of chemical, microbiological and packaging laboratories, together with a number of other test rooms. The equipment and library facilities are excellent.

The salary will be commensurate with the qualifications and experience of the successful candidate and will not be less than £2,000 per annum. There is a contributory Superannuation Fund and free Life Assurance cover. Luncheon Vouchers are provided.

Apply with full details of experience and personal particulars to the Administrative Manager (Ref. S.T.12), Wingate House, 93/107 Shaftesbury Avenue, London, W.1, marked "Private and Confidential."

DEPUTY CHIEF TSETSE OFFICER

UGANDA

Required to deputize for the Chief Tsetse Officer in the general administration of the Tsetse Control Division as well as being personally responsible for the planning and execution of individual schemes. Candidates, who should be nationals of the United Kingdom or Republic of Ireland whose permanent place of residence is in either of these countries, should possess a Degree in Zoology with postgraduate qualifications or experience in Entomology, preferably in insect ecology. They must have wide experience of tsetse survey and control operations in the field. An aptitude for general administrative work is essential. Salary £2,814 to £3,000 per annum (including Overseas Addition) plus 25 per cent terminal gratuity. Free Passages. Education Allowances. Quarters available. 21-27 month contract. Please apply for further details, giving full name, qualifications, experience and quoting Ref. RC 217/183/09 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE—
UGANDA

Applications are invited for:

Readership and 2 Lectureships in Anatomy.

Lectureship in Pharmacology. Candidates should hold a degree in Pharmacology, Physiology or Pharmacy.

Appointments on permanent terms, or on contract terms carrying 25 per cent gratuity (15 per cent if in F.S.S.U.). Salary scales (including expatriation allowances): for medically qualified candidates, Reader, £2,785 to £3,105 per annum; Lecturer, £1,600 to £2,400 per annum; for non-medically qualified candidates, Reader, £2,710 to £3,030 per annum; Lecturer, £1,550 to £2,350 per annum. Salary scales currently under review. Subsidized accommodation. Expatriate staff receive: education allowances; family passages; biennial overseas leave.

Detailed applications (six copies), naming three referees, by November 30, 1965, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom full details may be obtained.

UNIVERSITY OF BRISTOL

Applications are invited for the post of

LECTURER (GRADE II)

in ZOOLOGY, tenable from January 1, 1966, or such date in 1966 as may be agreed. Preference will be given to applicants with special interests in one of the following fields: invertebrate functional morphology, respiratory physiology or fish physiology, but the appointment may be made to a suitable candidate with other interests. Salary within the scale £1,400 to £2,505 per annum.

Further particulars may be obtained from the Registrar, University Senate House, Tyndall Avenue, Bristol 2, to whom applications should be sent by December 1.

WOOLWICH POLYTECHNIC

LONDON, S.E.18

DEPARTMENT OF CHEMISTRY

Applications are invited for the post of Lecturer in Analytical or Physical Chemistry. Teaching duties will be concerned with Analytical or Physical Chemistry for B.Sc. (Special) in Chemistry and for the Council for National Academic Award degree in Analytical Chemistry. Excellent facilities for research are available in the new laboratories and the person appointed will be expected to undertake research. Salary scale: £1,875 by £50 (2) by £55 (3) to £2,140 plus London allowance of £70.

Particulars and application form from Clerk to the Governors to be returned by November 26, 1965.

UNIVERSITY OF LIVERPOOL

Applications are invited for the post of Research Technician in the Department of Biochemistry for work in bacterial enzymology. Applicants should have qualifications (not necessarily a degree) or experience in microbiology, biochemistry or chemistry. Opportunity to study for higher qualifications and, in the case of graduates, for registration for the M.Sc. degree. Initial salary up to £750 per annum according to age, qualifications and experience.

Application forms may be obtained from the Registrar, University, Liverpool 3. Please quote Ref. 329/N.

Ferranti electronics

Vacancies have arisen at the Company's Wythenshawe (Manchester) Laboratories, which will offer the opportunity for interesting **Semiconductor Measurement and Test Gear Design** work on

INTEGRATED SEMICONDUCTOR CIRCUITS

Candidates should preferably possess a degree or Dip.Tech. in Physics, or Electrical Engineering, and experience of VHF and/or millimicrosecond pulse measurement techniques. Applications from holders of H.N.C. will also be considered if their experience is appropriate.

The modern laboratories are situated on the Cheshire boundary with easy access to pleasant rural areas.

Application forms may be obtained from T. J. Lunt, Staff Manager, Ferranti Limited, Hollinwood, Lancs. Please quote reference WAK.

NORTHERN POLYTECHNIC, HOLLOWAY, LONDON, N.7

Head of Department of Mathematics

The Governing Body invites immediate applications for the post of Head of the Department of Mathematics (Grade IV). The Northern Polytechnic is a Regional College and is an Institution of the University of London with recognized teachers.

The work of the Department is at present principally concerned with the Internal degree of B.Sc. (Special Mathematics) and B.Sc. (General) in Mathematics and Statistics and with postgraduate work leading to Internal M.Sc., M.Phil., and Ph.D. degrees. It is hoped that the new Head of Department will develop additional courses in branches of mathematics which have special relevance to the needs of industry and business.

Salary scale: £2,675 by £75 to £2,900, plus £70 London allowance.

Apply for form of application and further particulars.

R. H. CURRELL, F.C.A.,
Clerk.

BIOCHEMIST

£2,000 p.a.

International Group of Companies require a Biochemist with experience of utilization of animal glands and by-products to take charge of industrial biochemical Research and Development Department in London E.C.1 District.

Starting salary is likely to be at least £2,000 per annum and there is a Non-contributory Pension Scheme.

Applications, in confidence, to Box No. 868, T. G. Scott and Son, Ltd., 1 Clement's Inn, London, W.C.2.

Opportunities in TBA MATERIALS RESEARCH

Expansion of the Chemical Research Department of Turner Brothers Asbestos Co. Ltd. creates the following senior and graduate vacancies—

SENIOR PHYSICAL/INORGANIC CHEMIST—to lead a small team investigating the bulk and surface properties of silicate fibres. Applicants should have a good honours degree or equivalent and will probably be Ph.D. Preferred age 28–35 with several years research experience in inorganic or physical chemistry, ideally in surface chemistry of silicates. (Ref. : N.1.)

PHYSICAL/INORGANIC CHEMIST—required for the above team. Good honours degree or equivalent essential, research qualification or experience in relevant fields an advantage. (Ref.: N.2.)

POLYMER CHEMIST—required for a section investigating fundamental aspects of the reinforcement of polymers by inorganic fibres. Honours degree or equivalent preferred, degree plus A.P.I. ideal. Practical experience in polymerization, polycondensation reactions and relationship of physical properties to chemical structure, a distinct advantage. (Ref.: N.3.)

Attractive salaries are offered, commensurate with the status and calibre of the successful applicants and the Company operates a contributory pension scheme. The Chemical Research Department is part of a large Research Division, occupying pleasantly situated and well-equipped new laboratories.

Applications, giving full details of age, education, qualifications and experience, present and required salary, will be treated in strictest confidence and should be addressed to :

THE CHIEF PERSONNEL MANAGER,
TURNER BROTHERS ASBESTOS CO. LTD.,
P.O. BOX 40, ROCHDALE, LANCS.

T&N *A Turner & Newall Company*

FISONS PHARMACEUTICALS LIMITED

GRADUATE BACTERIOLOGIST

FISONS PHARMACEUTICALS LIMITED wish to appoint a graduate in bacteriology (or equivalent), preferably with a few years' experience, to the staff of the Microbiology Department of its Research Laboratories. The work involves various and interesting aspects of pharmaceutical microbiology and the assessment of new chemotherapeutic products. The successful candidate will also be responsible to the head of the department for the training and supervision of junior technical staff.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

THE NICHOLAS RESEARCH INSTITUTE LIMITED

Vacancies exist for the following :

BIOCHEMIST
PHARMACOLOGIST
TOXICOLOGIST

at the Biological Laboratories of the above Institute

The nature of the work will depend to some extent on the desires and capability of the successful applicants. Applicants should have a University Degree or equivalent qualifications.

The initial salary will be between £900 to £1,600 per annum.

Further information may be obtained from Dr. E. H. L. Harries, R/J/3, Biological Research Controller, Nicholas Research Institute Ltd., Wexham Place, Framewood Road, Wexham, Bucks.

PROPOSED UNIVERSITY OF BRADFORD

(Bradford Institute of Technology)

LECTURER OR ASSISTANT LECTURER IN PHYSICS

Applications are invited for the post of Lecturer or Assistant Lecturer in Physics. Candidates should be well-qualified physicists: experience of the application of physics in industry would be a strong recommendation. The successful candidate would be expected to contribute to the teaching and research in physics ; work is in progress in nuclear physics, magnetism and printed-character recognition. Considerable developments are planned in the fields of solid state physics and of materials science ; in the latter field there are particularly good opportunities for an electron microscopist. Salary within the range: Lecturer, £1,400 to £2,505 per annum ; Assistant Lecturer, £1,050 to £1,275 per annum. F.S.S.U., grant towards approved removal expenses.

Details of the post and application forms may be obtained from the Registrar, Bradford Institute of Technology, Bradford, 7.

CHEMICAL ENGINEER

HONDURAS

Applications are invited from candidates to assist in the development of a new project.

The Engineer will be responsible to the Development Bank and he must have had sufficient experience to be able to advise and assist the Government in the *use* of manufactured molasses, which is a rapidly expanding industry, for the production of rum, yeast, industrial alcohol and other liquors.

The appointment will be on contract for a period of approximately one year. Salary will be £3,200 per annum, subject to United Kingdom tax, plus a variable tax free overseas allowance currently ranging from £535 per annum to £1,225 per annum according to the domestic circumstances of the officer selected.

Free passages, medical attention, accommodation at low rental, generous leave and children's education allowances.

Apply for further details to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, Victoria, S.W.1.
giving full name, age and qualifications and quoting RC 288/80/01.

UNIVERSITY OF KENT AT CANTERBURY

APPOINTMENTS IN MATHEMATICS

Applications are invited for a number of appointments in Pure Mathematics, Applied Mathematics and Statistics from October 1, 1966. At least one appointment will be to a Readership or Senior Lectureship ; the others will be to Lectureships or Assistant Lectureships. Candidates in Pure Mathematics should have an interest in one of the following fields: theory of functions, functional analysis, algebra, algebraic geometry or topology. In Applied Mathematics present research and teaching interests include continuum mechanics, quantum field theory and elementary particle theory, but it is hoped to broaden these interests and applications are welcomed from candidates in other fields.

Further particulars and forms of application may be obtained from the Registrar, University of Kent at Canterbury, Canterbury. Completed applications should be received not later than Wednesday, December 1.

UNIVERSITY OF GLASGOW

CHAIR OF PHYSIOLOGY

Applications are invited for appointment to an additional Chair of Physiology for which an Ordinance has been promoted. This is a new appointment.

Applications (20 copies), should be submitted not later than November 26, 1965, to the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

UNIVERSITY OF THE WEST INDIES

Applications are invited for (a) Senior Lectureship or (b) Lectureship in the teaching of Science and, if possible, Mathematics, in Department of Education. Group research is undertaken and applicants should indicate their interest in this field. Salary scales: (a) £1,950 to £2,940 per annum; (b) £1,450 to £2,290 per annum. Child allowance. F.S.S.U. Housing allowance of 10 per cent of salary, or if available, university unfurnished accommodation at 10 per cent of salary. Family passages. Triennial study leave.

Detailed applications (six copies), naming three referees should be sent by November 27, 1965, by persons living in the Americas and Caribbean area to Registrar, University of the West Indies, Kingston 7, Jamaica, and by all others to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1. Further particulars may be similarly obtained.

ROYAL COLLEGE OF ADVANCED TECHNOLOGY
SALFORD
(Proposed University of Salford)
DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY
EXPERIMENTAL OFFICER IN SPECTROSCOPY

Applications are invited from persons with professional qualifications in electronics, physics or chemistry and appropriate experience, for appointment as Experimental Officer in the above department. The person appointed will assist members of staff in research in Electron Spin Resonance and other branches of spectroscopy in the new building for Nuclear Science and Technology. The successful candidate will be expected to take up duty as soon as possible. Salary scales: £920 to £1,340 (bar) to £1,555 a year, with superannuation. Placement according to qualifications and experience.

Applications for the post should be received within two weeks of the appearance of this advertisement, on the appropriate form of application, obtainable from the Secretary, Royal College of Advanced Technology, Salford, 5. Please quote reference CH/32.

RHODES UNIVERSITY
GRAHAMSTOWN, SOUTH AFRICA
PROFESSOR OF PHYSICAL CHEMISTRY

Applications are invited for the above-mentioned post from May 1, 1966. The salary scale is: R.5,000 by R.200 to R.6,000 per annum (£2,500 by £100 to £3,000 per annum). A vacation savings bonus, as in the Public Service is also payable. Previous experience and qualifications may be taken into account in determining the commencing salary. Membership of the University's medical aid and pension schemes is compulsory.

Full particulars and application forms may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in South Africa and London on November 30, 1965.

UNIVERSITY OF LIVERPOOL
IMMUNOCHEMISTRY UNIT (CANCER RESEARCH), DEPARTMENT OF BACTERIOLOGY

Applications are invited from Physical Chemists (Ph.D. or equivalent) interested in fundamental immunological cancer research for appointment as Research Assistant. Applications from immunologists, pharmacologists etc., with physico-chemical interests also welcome. Starting salary within the range £1,000 to £1,600 per annum, according to experience.

Applications, stating age, qualifications and experience should be received by the Registrar, The University, Liverpool 3, from whom further particulars may be obtained, as soon as possible, and not later than January 15, 1966. Please quote Ref. CV/330/N.

JUNIOR SCIENTIFIC OFFICER—BLOOD Transfusion Service. Successful applicant should hold B.Sc. Degree of a British University with main subject Bacteriology. Person appointed will take part in all activities of the Centre and be responsible for supervision of sterility testing and sterilization procedure. Research encouraged, projects available. Centre housed in new premises, equipment available. Whitley Council conditions. —Further information from the Director, Vincent Drive, Birmingham 15, by appointment. Applications to Secretary, 146 Hagley Road, Birmingham 16, naming three referees, by November 15, 1965.

British Rail

Assistant Director

Electrical Research Division

The Electrical Research Division of the British Railways Board is some 70 strong and the Assistant Director will be responsible for research on heavy current problems. This research utilises about 50% of the Division's resources and includes work on electric motors, overhead transmission systems and high-power switching. Research on light current problems is the responsibility of another Assistant Director.

Basic requirements are

- ☐ *A good Degree (or equivalent) in Electrical Engineering or Physics*
- ☐ *A successful personal record in research*
- ☐ *The ability to make effective scientific and technical contributions to a wide range of electrical engineering work*
- ☐ *A sound grasp of the management of applied research, backed by several years' experience of leading a research or development team*

The Railways Board plans to centralise mechanical, civil, chemical and electrical engineering research at a new technical centre with extensive design and development facilities to be built at Derby. In this new and stimulating environment, the scope of electrical research will certainly broaden and increase in effectiveness. Salary by negotiation upwards of £3,000 p.a. The usual superannuation benefits and travel facilities will apply. Please write, **by 15th November,** quoting Non.U.816 to Chief Management Staff Officer, Room 561,

British Railways Board
222 Marylebone Road, London NW.1

Ph.D. MICROBIOLOGY

R & D opening in basic and applied microbiology research for Ph.D. New position requires exceptional individual with originality and drive to handle challenging industrial fermentation problems. A ground floor opportunity in a new and growing field. Excellent San Diego, California location. Salary commensurate with ability and experience.

Submit résumé and salary requirements in confidence to: Personnel Manager, Kelco Company, 2145 East Belt Street, San Diego, California 92113.

AGRICULTURAL DIVISION

RESEARCH AND DEVELOPMENT STATION
JEALOTT'S HILL, NR. BRACKNELL, BERKS.

Librarian-in-Charge

A vacancy occurs for a suitably qualified woman to take charge of a scientific library—main subjects Chemistry and allied subjects, Entomology, Mycology, Botany and Agriculture—comprising some 6,000 books, 10,000 bound periodicals, together with reports, memoranda etc. The Library subscribes to some 700 periodicals.

Applicants must hold A.L.A. qualification or its equivalent, and have previous experience of library work. Ability to supervise and manage the day-to-day affairs of the library. Age range 25–40. 'O' or 'A' levels in one or more biological or chemical subjects and some acquaintance with the chemical and/or agricultural industries is desirable.

I.C.I. Pension Fund and Profit Sharing Schemes operated, together with progressive salary plan.

Write, giving brief details, to The Staff Officer,
PLANT PROTECTION LTD.,
Fernhurst, Haslemere, Surrey.

UNIVERSITY OF ALBERTA
DEPARTMENT OF CHEMISTRY

Applications are invited for the position of

PROFESSOR in Physical Chemistry

Candidates in the fields of Solid State, Structure of Liquids, Spectroscopy or Thermodynamics are favoured, but all applications will be considered. Starting salary according to qualifications, in the range $15,000 to in excess of $20,000. Facilities for research are excellent and generous support is obtained from Provincial and National sources. The Department has 30 members and is active in all the major divisions of chemistry.

Further particulars may be obtained from
Professor Harry E. Gunning, Head,
Department of Chemistry,
University of Alberta,
Edmonton, Alta., Canada.

AGRICULTURAL ASSISTANT

J. & J. Colman Limited, of Norwich, a member of the Reckitt & Colman group of companies, requires an Assistant for its Agricultural Section which is responsible for placing contracts for the growing of mustard seed and other crops, and for programmes of plant breeding and agronomic research. The Company manufactures a wide range of food products.

Candidates should preferably have either a degree or National Diploma in agriculture or some equivalent qualification. Since some experience in experimental work on arable crops is essential it is unlikely that the successful candidate will be under the age of 26 ; experience of practical farming would also be an advantage.

Starting salary will depend on age and experience. The Company operates a pension scheme and other benefits normally found in a large group.

Please apply with full particulars to the Personnel Director, J. & J. Colman Limited, Carrow Works, Norwich. NOR 75A.

UNIVERSITY OF QUEENSLAND
LECTURER IN INORGANIC CHEMISTRY

The University invites applications for the position of Lecturer in Inorganic Chemistry. An applicant should possess an honours degree in some branch of chemistry and if possible the degree of Doctor of Philosophy. He must have carried out some research. Applications are especially invited from anyone specializing in such fields of study as: co-ordination compounds ; compounds of boron, silicon or phosphorus ; π-complexes of transition metals, carbonyl chemistry ; or some other branch of modern inorganic chemistry. The salary range for Lecturer is £A2,400 by £A100 (7) to £A3,100 per annum. The University provides Superannuation similar to F.S.S.U., Housing Assistance, Study Leave and Travel Grants.

Additional information and application forms will be supplied upon request to the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in London and Brisbane on November 26, 1965.

BIOCHEMIST
UGANDA

The Government of Uganda have a requirement for a Biochemist to take charge of the Biochemistry Division of the Laboratory services located in Kampala and serving all parts of the country and all races. Most of the work originates from Mulago Hospital. Excellent library facilities and opportunities for research.

Candidates should possess an honours degree in Biochemistry or Associateship of the Royal Institute of Chemistry and have had at least two years postgraduate experience in medical biochemistry in a hospital laboratory. Candidates must also be nationals of and permanently resident in the United Kingdom or the Republic of Ireland.

Salary: £1,374 to £3,000 per annum, according to experience plus 25 per cent terminal gratuity. Free passages. Quarters available at low rental. 21 to 27 months contract.

Please apply for further particulars, giving full name to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 404, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF LANCASTER
SECOND CHAIR OF BIOLOGY

The University of Lancaster invites applications for a second CHAIR OF BIOLOGY from persons whose main interests lie in zoology or physiology.

Further particulars may be obtained from the Secretary, University of Lancaster, Bailrigg House, Lancaster, to whom applications (a single copy) should be sent so as to arrive by December 31, 1965.

THE LABORATORIES BELFAST CITY HOSPITAL
POST OF BIOCHEMIST

Applications are invited for the post of Non-Biochemist (Basic Grade) in The Laboratories, Belfast City Hospital. Applicants should hold a Science Degree in Chemistry or Biochemistry of a university of England, Wales, Scotland or Ireland, or the Associateship or Graduate Membership of the Royal Institute of Chemistry. There is a principal and a senior biochemist in post. The salary will be that applicable in the National Health Service.

Application forms and further particulars may be obtained from the Secretary, Belfast City Hospital, Belfast 9, to whom completed applications should be submitted before December 6.

MANCHESTER EDUCATION COMMITTEE

JOHN DALTON COLLEGE OF TECHNOLOGY

The Science Research Council is prepared to offer to suitable candidates two Research Assistantships tenable at Metallurgy Department, John Dalton College of Technology to assist in a research programme dealing with (a) Fibre Metallurgy; (b) stress corrosion cracking. The value of these awards will be £500 plus two (yearly) increments of £50 and will be tenable for three years. Applicants should possess a Higher National Certificate in Metallurgy or an equivalent qualification.

For further details, write as soon as possible to Dr. H. J. Bray, Head of Metallurgy Department, John Dalton College of Technology, Chester Street, Manchester 1.

UNIVERSITY OF BRISTOL

DEPARTMENT OF MATHEMATICS

Applications are invited for the post of

LECTURER (Grade II)
in
MATHEMATICS
(PURE OR APPLIED)

In the Department of Mathematics tenable from August 1, 1966, or such earlier date as may be convenient.

Further particulars may be obtained from the Registrar, University Senate House, Bristol, 2, to whom applications should be sent by November 30, 1965.

UNIVERSITY OF LANCASTER

DEPARTMENT OF BIOLOGY

Applications are invited for posts of ASSISTANT LECTURER or LECTURER (exceptionally SENIOR LECTURER) with special interest in the following fields: (i) microbiology of natural soils; (ii) quantitative analysis of the physical nature of the environment of plants and animals; (iii) biochemistry; (iv) genetics of micro-organisms; (v) methodology of taxonomy.

The salary scales are: Assistant Lecturer, £1,050 by £75 to £1,275; Lecturer, £1,400 by £85 to £2,505; Senior Lecturer, £2,450 by £100 to £3,150 (or £3,250 in exceptional cases).

Further particulars may be obtained from the Secretary, University of Lancaster, Bailrigg House, Lancaster, to whom applications should be sent not later than December 31, 1965.

DEPARTMENT OF RADIOTHERAPEUTICS

UNIVERSITY OF CAMBRIDGE

Technical Assistant required to work with a whole-body radioactivity counter used in clinical research. Preference will be given to applicants having some experience in radioactive counting techniques.

Remuneration under University Grade "T" regulations within the scale £796 to £1,068 per annum depending on qualifications and experience.

Applications, with names of two referees, to the Secretary, Department of Radiotherapeutics, Addenbrooke's Hospital, Cambridge.

APPLICATIONS ARE INVITED FOR RESEARCH posts in the following subjects: Primatology, Comparative Osteology and Palaeolithic Prehistory. Applicants should have University degree in appropriate subjects and application for study of primate disease by medical or veterinary candidates would be considered. Interviews will be arranged London last week November.—Apply by airmail, Dr. Leakey, P.O. Box 30239, Nairobi, Kenya.

FISONS PHARMACEUTICALS LIMITED

MICROBIOLOGIST

FISONS PHARMACEUTICALS LIMITED wish to appoint a Microbiologist, preferably with some experience, to the staff of the Microbiology Department of its Research Laboratories. The work involves various and interesting aspects of pharmaceutical microbiology, including production control and the assessment of new chemotherapeutic compounds.

Candidates (male or female) should possess at least the Intermediate I.M.L.T. or be taking the H.N.C. course in Applied Biology with preference for bacteriology.

The department is well equipped and is part of a vigorous and expanding research organization based in a pleasant rural district.

The Company is a member of the Fisons Group of Companies and Group Contributory Pension and other welfare schemes operate.

The Company works a five-day week and conditions of work are excellent. Three weeks annual holiday. Canteen available.

Applications should be sent to the Research Personnel Assistant, Fisons Pharmaceuticals Limited, Holmes Chapel, Cheshire.

CAPE

THE CAPE ASBESTOS GROUP OF COMPANIES

SENIOR RESEARCH CHEMIST

A new and challenging position has been created in the Research and Development Laboratory of Cape Building Products Limited, Uxbridge, for a chemist to take charge of the Research Section. The company's products are mainly based on lime-silica reaction and the candidate should have several years appropriate experience which may have been gained in concrete, cement or allied industries.

Familiarity with pilot plant and perhaps also full scale plant operation will be a distinct advantage. Suitable candidates in their early 30's will be expected to provide evidence of ability to head a team and take full responsibility for a wide variety of projects including research on lime-silica, development of new products from basic to full scale production stages, and service on research projects to the production departments. The appointment is at a Senior Staff level with responsibility to the Company's Technical Manager.

General conditions of service are excellent and the starting salary will compete favourably with current rates in relation to the responsibilities of the post.

Applications will be accepted in complete confidence. They should include a brief personal history with indication of present and/or required salary and be addressed, quoting ref.: W.6553, to :—

The Group Personnel Manager
THE CAPE ASBESTOS COMPANY LIMITED
114, Park Street, London, W.1.

H.M. INSPECTORS OF SCHOOLS

SCOTTISH EDUCATION DEPARTMENT

2 PENSIONABLE posts for men or women, preferably between 30 and 45, as Inspectors of:

(i) BIOLOGY (ii) PRIMARY EDUCATION

QUALIFICATIONS: Good teaching experience. Also for (i) First- or second-class honours degree in biological subject; for (ii) candidates must be certificated teachers with good experience in primary schools, and knowledge of modern developments in curriculum and method.

SALARY: £2,309 to £3,457. Starting salary may be above minimum. Promotion Prospects.

WRITE: Scottish Education Department (Establishment), St. Andrew's House, Edinburgh, 1, for application form. Closing date November 19, 1965.

UNIVERSITY OF EAST ANGLIA

SCHOOL OF MATHEMATICS AND PHYSICS

Applications are invited for posts of Reader, Senior Lecturer, Lecturer or Assistant Lecturer in Physics from October 1, 1966. The stipends will be on the scale: Reader, £2,550 by £100 to £3,250; Senior Lecturer, £2,450 by £100 to £3,150; Lecturer, £1,400 by £85 to £2,505; Assistant Lecturer, £1,050 by £75 to £1,275, plus F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C, with whom applications (one copy only), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 20, 1965.

UNIVERSITY OF BELFAST

CHAIR OF PHYSICS

The Senate of The Queen's University of Belfast invites applications for the Chair of Physics in the Department of Physics in the School of Physics and Applied Mathematics from October 1, 1966. Applications should be received by December 15, 1965.

Further particulars may be obtained from G. R. Cowie, M.A., LL.B., J.P., Secretary.

Allen & Hanburys Ltd. Research Division, Ware, Herts.

Applications are invited from suitably qualified scientists for the position of Scientific Officer in the Medical Department of the Research Division. The main duties of the successful candidate will be to ensure that clinical trials of new medicaments proceed in an efficient, orderly manner. The applicants should preferably have a background in physiology, biochemistry, medicinal chemistry, pharmacology or pharmacy, and may have a higher degree. The ability to order, assess and present experimental or clinical data will be required, although candidates might not already possess this experience. Since the position will involve visits to hospitals throughout the country, a company car will be provided.

Applications, giving brief details of age, qualifications and experience, should be addressed to the Personnel Manager, quoting reference no. MD4.

LIVERPOOL EDUCATION COMMITTEE

LIVERPOOL REGIONAL COLLEGE OF TECHNOLOGY
BYROM STREET, LIVERPOOL, 3
Principal: S. A. J. Parsons, B.Sc.(Econ.), M.I.Mech.E., M.I.Prod.E., M.B.I.M.

DEPARTMENT OF CHEMISTRY AND BIOLOGY

Applications are invited from well-qualified candidates for: Post F/66S, Senior Lecturer in Organic Chemistry to begin January 1, 1966, or as soon as possible thereafter. Candidates must be prepared to teach organic chemistry to Honours B.Sc. level and to initiate and direct research. Teaching or industrial experience is desirable. Salary: £2,140 to £2,380 per annum.

Application forms and further particulars can be obtained from the Director of Education, Education Offices, 14 Sir Thomas Street, Liverpool, 1, on receipt of an addressed foolscap envelope. Return completed forms to the Principal of the College not later than November 22, 1965.

THOMAS ALKER,
Town Clerk.

UNIVERSITY OF LEEDS

Applications are invited for three posts of Lecturer or Assistant Lecturer in The Department of Physical Chemistry (Head of Department: Professor Peter Gray) at salaries on the scale £1,050 by £75 to £1,275 for an Assistant Lecturer or £1,400 by £85 to £2,505 (efficiency bar £2,165) for a Lecturer. For one post preference may be given to candidates with a special interest or experience in Chemical Physics or Theoretical Chemistry.

Applications (three copies), stating age, qualifications and experience and naming three referees, should reach the Registrar, the University, Leeds, 2 (from whom further particulars may be obtained) not later than February 1, 1966. The particular attention of overseas candidates is drawn to this closing date, which is intended to give them ample opportunity to apply.

UNIVERSITY OF GLASGOW

CHAIR OF BIOCHEMISTRY

Applications are invited for appointment to an additional Chair of Biochemistry for which an Ordinance has been promoted. This is a new appointment.

Applications (20 copies), should be submitted not later than November 26, 1965, to the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

CLARKSON COLLEGE OF TECHNOLOGY

POTSDAM, NEW YORK, U.S.A.

GRADUATE RESEARCH AND INSTRUCTIONAL ASSISTANTSHIPS for M.S. and Ph.D. candidates in CHEMICAL ENGINEERING available for February and September, 1966. Stipends $2,400 to $4,100 plus tuition for 12 months. Research in adsorption, crystallization heat transfer, reverse osmosis, mass transfer, reaction kinetics, boundary layer and other topics.

Applicants may contact: H. L. Shulman, Dean of the Graduate School, Clarkson College of Technology, Potsdam, New York, U.S.A.

VICTORIA UNIVERSITY OF WELLINGTON

NEW ZEALAND

LECTURER IN BOTANY

The Council of Victoria University of Wellington invites applications from suitably qualified persons for the position of Lecturer in the Department of Botany. This is a new post and is not being allocated in advance to a particular branch of the subject. The salary for a Lecturer will be £1,300 per annum, rising to £2,000—with a bar—then on recommendation to £2,200 per annum, initial salary being determined in accordance with qualifications and experience. Approved fares to Wellington will be allowed for the appointee and his family, together with actual removal expenses within specified limits.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in New Zealand and London on November 30, 1965.

ANIMAL VIRUS RESEARCH INSTITUTE, Pirbright, Surrey, requires an Assistant Experimental Officer/Experimental Officer to take charge of the maintenance and operation of a Siemens Electron Microscope Model Elmiskop 1. The successful candidate will be required to participate in a programme of work on the structure of animal viruses. Salary scale, A.E.O. £983 (at age 26) to £1,201; E.O., £1,319 to £1,675. Starting salary depending on age, qualifications and experience. Superannuation.—Applications to Secretary.

NORTHERN POLYTECHNIC

HOLLOWAY, LONDON, N.7

The Governing Body invites immediate applications for the post of Lecturer in the Department of Physics. The work of the Department is all of Degree level with a high proportion of post-graduate work. The Lecturer should be well qualified in physics. Experience in some branch of electronics would be an advantage. A sufficiently experienced candidate might be considered for a Senior Lectureship.

Salary scales
Lecturer: £1,875 by £50 to £1,975 by £55 to £2,140 plus £70 London Allowance.
Senior Lecturer: £2,140 by £60 to £2,380 plus £70 London Allowance.

Apply for form of application and further particulars.

R. H. CURRELL, F.C.A.,
Clerk.

UNIVERSITY OF LEEDS

Applications are invited for appointment as ASSISTANT LECTURER in Pharmacological or Pharmaceutical Chemistry in THE DEPARTMENT OF PHARMACOLOGY. Candidates should be graduates in chemistry or pharmacy. Duties may include teaching in degree courses in pharmacy and pharmacology.

Salary on the scale £1,050 by £75 to £1,275. Initial appointment at any point on the scale. Appointment to date from January 1, 1966, or as soon as possible thereafter.

Applications (three copies), stating age, qualifications and experience and naming three referees, should reach the Registrar, the University, Leeds 2 (from whom further particulars can be obtained) not later than November 27, 1965.

IRRIGATION AGRONOMIST

BECHUANALAND

Required to test and demonstrate irrigation on the more promising soil types or areas indicated by the Soil Surveyor, and to be responsible for instigating and initiating water use trials on either areas of existing irrigation or on such new investigational or pilot projects as prove desirable and possible.

Candidates must hold a degree in agriculture with postgraduate training in field experimentation. A sound knowledge of evapo-transpiration, soil-plant moisture relations and of methods of water application for crop production is essential.

Salary range £1,700 to £2,200 a year subject to British income tax, plus a tax free overseas allowance of either £235 (single), £600 (married unaccompanied) or £720 (married accompanied) a year. Passages provided. Education allowances. Two-year contract.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should write for further details, giving full name and brief particulars of qualifications and experience, quoting RC 213/19/08 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF QUEENSLAND

PHARMACOLOGIST OR BIOCHEMIST

A vacancy exists in the Department of Physiology for a Research Officer Grade I (Postdoctorial) in pharmacology or biochemistry to investigate the pharmacology or biochemistry of oxytocic substances in blood extracts. Salary range £A2,400 to £A3,100. Alternatively applications from experienced investigators in pharmacology or biochemistry would be considered for a position of Research Officer Grade II. Salary range £A1,500 to £A2,150 according to experience and qualifications. Appointment for 1966, and extension subject to grant being available.

An appointee from the United Kingdom would be nominated under the assisted passage scheme.

Applications, stating age, qualifications and experience should be forwarded to the Registrar, University of Queensland, Brisbane, Australia, with whom applications close on November 28, 1965.

C. J. CONNELL,
Registrar.

BIOLOGIST
TANZANIA

A biologist is required to investigate the ecology of game animals in the woodland areas. Candidates (between 24 to 35 years) should hold an honours degree in Zoology with postgraduate experience in animal ecology. Salary £1,281 to £2,757 per annum, plus 25 per cent terminal gratuity. Passages provided. Education allowances. Furnished accommodation. Generous home leave. Appointment on contract for one or two tours of 21 to 27 months each tour.

Candidates, who should be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting Ref.: RC 275/173/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

FISHERIES OFFICER
TURKS AND CAICOS

A Fisheries Officer is required to investigate the fisheries potential of the Islands, markets for sea products and to organize a Fisheries co-operative at Salt Cay. Preference will be given to candidates with a university degree. Salary £2,275 plus 20 per cent terminal gratuity. Passages provided. Free accommodation. Free medical attention. Education allowances. Three-year contract.

Candidates, who should normally be nationals of the United Kingdom or the Republic of Ireland, should apply for further details, giving full name and brief particulars of qualifications and experience, quoting reference RC.289/182/01 to:

Appointments Officer,
Ministry of Overseas Development,
Room 301, Eland House,
Stag Place, London, S.W.1.

UNIVERSITY OF EAST ANGLIA
SCHOOL OF MATHEMATICS AND PHYSICS

Applications are invited for a number of posts as Lecturer or Assistant Lecturer in Mathematics in the School of Mathematics and Physics from October 1, 1966. For one of the posts some preference may be given to candidates with research interests in algebra. The stipends will be on the scale: Lecturer, £1,400 by £85 to £2,505; Assistant Lecturer, £1,050 by £75 to £1,275, plus F.S.S.U. benefits.

Further particulars may be obtained from the Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C, with whom applications (one copy only), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 20, 1965.

GRADUATE, PREFERABLY IN A BIOlogical science, required for full-time or part-time work at the Medical Research Council's Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Glamorgan. The main duties would be administration of a small scientific and medical library, making extensive use of inter-library loans; occasional bibliographic work; and assisting the scientific staff of the Unit (which includes physiologists, pathologists, physicists, chemists, and mathematicians) by investigating useful sources of information in specific biological fields. This would involve not only searches in the relevant literature but also finding and making contact with scientific workers with relevant knowledge. The qualities needed would be initiative, curiosity and common sense, with a sound background of scientific training but not necessarily outstanding academic ability. The salary offered would be subject to negotiation with the successful candidate.—Apply to the Administrative Officer at the above address, giving full details of age, qualifications and experience.

UNIVERSITY COLLEGE LONDON

Full-time RESEARCH ASSISTANT required in Department of Anatomy (Head of Department: Professor J. Z. Young) for work on the electron-microscopy of cells in tissue culture. Previous experience in either of these techniques is not essential, but would be an advantage. Duties to begin as soon as possible. Salary about £1,000 per annum, depending on qualifications and experience.

Applications should be sent to the Secretary, University College London, Gower Street, W.C.1, from whom further particulars may be obtained.

STATISTICIAN

Applications are invited from men and women for the position of Statistician in the Association's Mathematics Section. This Section is concerned with the formulation and solution of a wide range of problems, arising principally from the field of electrical engineering. Much of the work is of an original nature, with the possibility of publication in the technical press.

A First or an Upper Second Class Honours Degree in Statistics, or in Mathematics with Statistics, is essential. A knowledge of a simplified Autocode language, such as Mercury Autocode or Algol, would be an advantage.

This appointment carries an attractive salary which will be initially negotiated according to qualifications and experience.

The Association has excellent conditions of service including a contributory Pension Scheme, Staff Restaurant facilities and a Sports and Social Club.

If you are interested in the above appointment please write to or telephone:

P. L. Bye, Personnel Officer,
The Electrical Research Association,
Cleeve Road, Leatherhead, Surrey.

Telephone Leatherhead 4151.

Cementation International Civil and Mining Contractors and Engineers

BASIC RESEARCH

Well qualified Physicists and Chemists with research experience are required to expand teams engaged in basic and applied research related to the wide range of specialist engineering and mining activities of the Cementation Group of Companies. We are seeking men with initiative, capable of taking responsibility for projects, to fill permanent and responsible posts. Full recognition is given to individual work and ideas and the publication of original work is encouraged.

PHYSICISTS

to work on interesting and varied problems associated with flow of fluids, properties of materials, acoustics, and transmission of vibration.

CHEMISTS

Ph.D.s' to invent, investigate, and develop novel materials, such as hydrogel systems for use in the consolidation and impermeabilisation of strata The work encompasses all three main branches of chemistry.

Applicants should apply in writing to:
Dr. R. A. Scott,
The Cementation Company Limited,
Central Research Laboratories,
Denham Way, Maple Cross,
Rickmansworth, Herts.

GRADUATE PHYSICIST REQUIRED FOR responsible post with a soundly established company of optical instrument manufacturers: rapid advancement is possible for the selected applicant who proves he is the man for the job. Accommodation in the form of a self-contained flat at an economic rent in pleasant surroundings and within easy reach of the factory is available if required.—If you wish to be considered for this exceptional opportunity, please write with relevant particulars to the Technical Director, Ross Limited, 4 Clapham Common, North Side, London, S.W.4.

BRADFORD INSTITUTE OF TECHNOLOGY
(The Proposed University of Bradford)

Applications are invited for the post of Professor of Statistics and Operational Research. Salary scale within the range of that for university professors. The successful candidate will be responsible for research and development in his subject.

Further details and application forms from the Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH CHEMISTS

Able young Post-doctoral Organic Chemists, required to work in a modern well equipped laboratory on the isolation and synthesis of Pharmacologically active compounds; experience in natural product work is desirable.

Apply to the Managing Director, Biorex Laboratories Ltd., Research Division, Apsley House, 198 City Road, London, E.C.1.

UNIVERSITY OF ALBERTA

DEPARTMENT OF DAIRY AND FOOD SCIENCE
EDMONTON, ALBERTA, CANADA
RESEARCH ASSOCIATE IN DAIRY TECHNOLOGY

Applications are invited for the above post to lead a small research team to undertake investigation in butter and fluid milk. The position will be for a period of two years in the first instance and may be renewed on a permanent basis thereafter. The salary will be in the range of $8,200 to $12,000 per calendar year, depending on the qualifications and experience of the applicant, who should preferably have a Ph.D. or the equivalent and research experience.

The successful applicant will work in the Department of Dairy and Food Science and be responsible to the Head of the Department. He will have the facilities of the Department at his disposal, and will enjoy the social privileges accorded to academic staff, but will not (at least during the first two years) be entitled to join the University Superannuation Scheme. It is hoped that the successful applicant will be able to take up the position early in 1966. Applications should be sent to the Head, Department of Dairy and Food Science, University of Alberta, Edmonton, Alberta, and should include personal data, academic qualifications and experience, a list of published work, together with transcripts, names of four referees and a recent photograph.

PROPOSED UNIVERSITY OF BRADFORD

(BRADFORD INSTITUTE OF TECHNOLOGY)

A Technician/Senior Technician is required in the Department of Biological Sciences. Duties involve assistance with Teaching Laboratories and relating classes, researches in biochemistry, controlled environment work and instrumentation and with the development of bioassay and culture techniques. Further information obtainable from the Professor of Biological Sciences. Salary according to qualifications and experience in range: Senior Technician, £840 to £1,040; Technician, £600 to £840, with an increment of £30 or £50 in either case for approved qualifications.

Application forms from the Secretary, Bradford Institute of Technology, Bradford 7, to whom they should be submitted as soon as possible.

ESSEX

MID-ESSEX TECHNICAL COLEGE AND SCHOOL OF ART
VICTORIA ROAD SOUTH, CHELMSFORD

Required January, 1966, Assistant Lecturer Grade B in Biological Subjects in rapidly developing section of the Science Department, for "A" Level Botany and the new O.N.D./O.N.C. Courses. Interest in Applied Biology and/or teaching experience desirable. Salary scale: £975 to £1,625. Higher scales may apply if training and graduate qualifications are in accordance with the Further Education Salaries Report requirements. Increments above the scale minimum allowed for previous teaching, war/national service and approved relevant industrial experience. Assistance with removal expenses may be given.

Further particulars and application form from the Principal.

POST-DOCTORAL BIOCHEMIST (LECturer grade) required for research group working on isoenzymes in normal and malignant tissues. (British Empire Cancer Campaign for Research).—Apply to Professor D. N. Baron, Department of Chemical Pathology, Royal Free Hospital, Gray's Inn Road, London W.C.1.

APPLICATIONS ARE INVITED FOR THE post of Assistant Lecturer in Chemical Metallurgy. The work involves teaching at undergraduate and postgraduate levels and research on metallurgical reactions in aqueous systems. Research facilities are exceptionally good and there is plenty of opportunity in this developing field. Salary in scale £1,110 by £75 to £1,335 plus F.S.S.U., according to qualifications and experience. Applicants should hold qualifications in Chemistry, Metallurgy or Chemical Engineering.—They should apply with full curriculum vitae to Professor F. D. Richardson, Department of Metallurgy, Imperial College, Prince Consort Road, London, S.W.7.

NATIONAL BLOOD TRANSFUSION SERVICE

CHURCHILL HOSPITAL
HEADINGTON, OXFORD

Applications are invited for the post of JUNIOR SCIENTIFIC OFFICER in the Laboratories at this Centre. Candidates should possess a B.Sc. degree or equivalent degree of a University of the United Kingdom, in appropriate subjects. Salary £830 to £1,175 (probationary period) or £935 to £1,175 for entrants with first- or second-class honours degree. Post-probationary scale £1,010 to £1,610. Paid leave three weeks rising to four weeks per annum.

Applications, stating qualifications, age and experience, should be addressed to the Administrative Officer, Regional Transfusion Centre.

MENTAL RETARDATION RESEARCH

Organizer, Graduate level, required to collect and collate current information on research into Mental Retardation, and to develop library and other information services under the general guidance of a panel of specialists. Full-time secretarial assistance. Salary according to qualifications and experience.

Further particulars and application form may be obtained from: The Secretary, Research Advisory Panel, 5 Bulstrode Street, London, W.1.

BATTERSEA COLLEGE OF TECHNOLOGY

PROPOSED UNIVERSITY OF SURREY

DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING

Applications are invited for the post of Lecturer with a research bias and who has a Physics/Electronics background.

The salary will be at an appropriate point on the scale for University Lecturers, £1,400 to £2,505 per annum plus £60 London allowance.

Applications (no forms issued), giving the names of not more than three referees, should be sent to the Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 by November 19.

BATTERSEA COLLEGE OF TECHNOLOGY

PROPOSED UNIVERSITY OF SURREY

Applications are invited for the post of READER in APPLIED MATHEMATICS (vacancy existing through appointment of present holder to a Chair). Candidates should preferably, but not necessarily, be qualified in a branch of classical applied mathematics.

Salary within the scale £2,400 per annum to £3,000 (bar) to £3,250 per annum plus £80 London Allowance. Assistance towards removal expenses will be considered. The post will be superannuated under F.S.S.U. conditions.

Further particulars may be obtained from the Secretary of the College, Battersea Park Road, London, S.W.11, to whom applications (no forms issued), giving the names of not more than three referees should be sent by November 30.

The College is to have University status in the near future and plans are now well in hand for a move to a new site at Guildford.

UNITED NEWCASTLE UPON TYNE HOSPITALS

Probationer Biochemist required for duties in the Department of Clinical Biochemistry. This is a joint University/Teaching Hospital department and a considerable amount of research work is undertaken.

Candidates, who must possess an appropriate science degree will serve a probationary period before appointment to basic grade. Salary scale £830 to £1,175 per annum; after probationary period, £1,010 to £1,610 per annum.

Whitley Council conditions of service applicable.

Applications, giving full details of age, education, qualifications and experience (if any) with names of two referees, to the House Governor and Secretary, Royal Victoria Infirmary, Newcastle upon Tyne, 1.

KENT AND CANTERBURY HOSPITAL

CANTERBURY, KENT
(318 BEDS)

Applications are invited for the post of Principal Physicist to the Physics Department of the Kent and Canterbury Hospital Radiotherapy Centre. Extensive medical isotopes department attached and facilities for research and development projects are available and encouraged. Suitably qualified candidates must have a wide experience of Hospital Physics. Salary scale commencing at £2,255 rising to a maximum of £2,900 per annum.

Applications, together with the names of two referees, to be addressed to the Hospital Secretary at the above Hospital.

LONDON HOSPITAL MEDICAL COLLEGE

(UNIVERSITY OF LONDON)
DEPARTMENT OF PHARMACOLOGY

Applications are invited for the appointment of Lecturer in Pharmacology. Duties will include teaching to pre-clinical students and supervision of those undertaking Special B.Sc Courses, and ample time will be available for research. Medical qualification preferable, but not essential. Initial salary within the scale £1,805/£2,505 plus £60 London Allowance per annum, together with superannuation under the F.S.S.U.

Applications, two copies, together with the names of two referees, to the Secretary, The London Hospital Medical College, Turner Street, London, E.1, within twenty-one days.

UNIVERSITY OF NOTTINGHAM

LECTURESHIP OR SENIOR LECTURESHIP IN AUTOMATIC CONTROL

Applications are invited for this Lectureship or Senior Lectureship within the Department of Electrical and Electronic Engineering from applicants who should have had industrial and/or research experience in the control field. Salary within the range £1,400 to £2,505 or £2,450 to £3,150 with membership of the F.S.S.U.

Conditions of appointment and form of application to be returned by November 26, 1965, from the Registrar.

UNIVERSITY OF GLASGOW

LECTURESHIP IN MATHEMATICS

Applications are invited for a Lectureship in Mathematics from persons qualified in Pure or Applied Mathematics. Salary scale: £1,400 to £2,505 per annum. Initial salary according to experience and qualifications. F.S.S.U.

Applications (eight copies), should be lodged, not later than December 11, 1965, with the undersigned from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of the University Court.

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF MANITOBA

WINNIPEG, CANADA
POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral fellowship for research in the field of Aquatic Ecology. The appointment is for a twelve month period. The salary is $6,000 tax free with an additional $1,500 for light teaching duties. Applicants with interests in the population dynamics of fishes, zooplankton and benthic animals will receive preference.

Applications, giving academic qualifications, details of research experience and the names of three referees, should be sent to the Head of the Zoology Department, Winnipeg 19, Manitoba, Canada.

MEDICAL RESEARCH COUNCIL

TRAVELLING FELLOWSHIPS IN MEDICINE AND MEDICAL SCIENCE

The Medical Research Council invite applications for the following Travelling Fellowship for the academic year 1966/67.

DOROTHY TEMPLE CROSS RESEARCH FELLOWSHIP

The object of these Fellowships is to give special opportunities for study or research to suitably qualified United Kingdom subjects of either sex, possessing suitable medical or scientific qualifications, intending to devote themselves to the advancement by teaching or research of the curative or preventive treatment of tuberculosis in all or any of its forms or to increase knowledge of diseases of the lung. The Fellowships will, as a rule, be awarded to candidates who wish to make their studies or inquiries elsewhere than in the United Kingdom. There are no precise age limits but awards will be made to candidates under the age of 28 and over 40 only in exceptional circumstances.

Fellowships tenable in the United States will carry a stipend of $6,000 per annum and where appropriate an allowance of $500 per annum will be payable for the fellow's wife and each dependent child. In addition, an allowance will be made for travelling and other approved expenses.

Further particulars of these awards and forms of application may be obtained from the Secretary, Medical Research Council, 20 Park Crescent, London, W.1, to whom completed forms should be returned by not later than January 1, 1966.

THE ROYAL SOCIETY

JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1966

The Council of the Royal Society is prepared to receive applications for the above research appointment, which is open to male British graduates under the age of 35 and is to be used for the encouragement of travel and work in oceanography or limnology. This award, which is to be taken up during 1966 and may be held for periods from three months to one year, will this year have the maximum value of £1,600 per annum and will be adjusted according to the qualifications of the successful candidate and the place and length of time of the research proposed. Applications are especially invited from experienced workers in the fields of oceanography and limnology, as well as from those in the fields of zoology, botany, geology, applied mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment.

It is hoped that the John Murray Travelling Student will try to gain practical experience of deep-sea oceanography, as initiated by the Challenger Expedition, though this is not a condition of the award which is available also for limnology. Assistance can be given when opportunities arise to holders of studentships to sail in oceanographic research ships, and help can be given in arrangements for visits and work at suitable institutions and research stations abroad.

Applications, which should be received not later than December 31, 1965, should be made on forms to be obtained from the Executive Secretary, The Royal Society, Burlington House, Piccadilly, London, W.1.

UNIVERSITY OF EXETER

DEPARTMENT OF CHEMISTRY

Applications are invited from suitably qualified candidates (a Ph.D. or its equivalent) for a Science Research Council Fellowship for work on the stereochemistry of reactions of some keto-steroids. The post is for two years and the salary will be £1,125 for the first year and £1,200 for the second year, with F.S.S.U. benefits. The successful applicant will be expected to take up the appointment by January 1, 1966, but an earlier starting date will be possible.

Applications (three copies), with the names of three referees, should be sent to the Secretary of the University, Northcote House, The Queen's Drive, Exeter.

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF ELECTRONICS RESEARCH STUDENTSHIP

Applications for the above post are invited from suitably qualified Electronic or Electrical Engineers or physicists interested in research in the field of Digital Frequency Modulation. It is anticipated that the work will last three years. Previous experience in this field is not essential. The successful applicant will be expected to register for a higher degree. The salary will correspond to that given under an S.R.C. award.

Applications should be sent to Professor G. D. Sims, not later than November 30, 1965.

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF METALLURGY RESEARCH FELLOWSHIP

Applications are invited for the post of Research Fellow in the Science Research Council Research Group on the Structure and Properties of Alloy Steels under the direction of the Professor of Physical Metallurgy (R. W. K. Honeycombe). Candidates should possess a Ph.D. in Metallurgy or Physics or equivalent qualifications together with some research experience in physical metallurgy or metal physics. Experience with modern electron microscopic or X-ray crystallographic methods would be advantageous. The appointment is in the first instance for three years. Salary in the range £1,400 to £1,740 with F.S.S.U. provision.

Further particulars may be obtained from the Registrar, to whom applications (three copies) should be sent by November 14, 1965.

UNIVERSITY OF TORONTO

DEPARTMENT OF CHEMISTRY POSTDOCTORAL FELLOWSHIPS

One or two postdoctoral fellowships available for experimental and theoretical research on kinetics of gas reactions, especially reactions at high temperatures in shock waves and detonations. Current topics include unimolecular and chain reactions, energy transfer and ionization processes, non-unidimensional phenomena in gas detonations. Experimental studies by time-resolved mass spectrometry of reactions in shock waves; high speed photography; measurements of shock density profiles. Excellent computer facilities available. Salary not less than $6,000 tax free plus possible assistance with travel costs.

Apply to Professor J. E. Dove, Department of Chemistry, University of Toronto, Toronto 5, Ontario, Canada.

LECTURES AND COURSES

CHELSEA COLLEGE OF SCIENCE AND TECHNOLOGY

MANRESA ROAD, LONDON, S.W.3 DEPARTMENT OF GEOLOGY PALAEO-ECOLOGY COURSE

A course in Palaeo-ecology, consisting of ten 2-hour periods, will be given in the Department by Dr. D. V. Ager, Reader in Geology, Imperial College, on Thursday evenings, 6 to 8 p.m., commencing January 13, 1966. The course will include lectures and discussion periods, practical work and one week-end field-class; it is intended for advanced and postgraduate students who have taken courses in morphological palaeontology.

Further particulars from the Head of Department.

BRADFORD INSTITUTE OF TECHNOLOGY

(Proposed University of Bradford) DEPARTMENT OF MATHEMATICS M.SC. IN SOLID MECHANICS

In the academic year 1966/67, a twelve months' course in Solid Mechanics will be held, leading to the degree of M.Sc. Applications are invited from candidates holding a good degree or Diploma in Technology, in Mathematics or other appropriate subject, and from students in their final year at college or university.

Inquiries should be made to the Registrar, Ref. 84X/X, Bradford Institute of Technology, Bradford 7.

UNIVERSITY OF LONDON: A COURSE of three lectures entitled "The Role of Efference in Visual Perception" will be delivered by Professor Leon Festinger (Stanford) at 6 p.m. on November 17, 18 and 19 at University College (Anatomy Theatre), Gower Street, W.C.1. Admission free, without ticket.—James Henderson, Academic Registrar.

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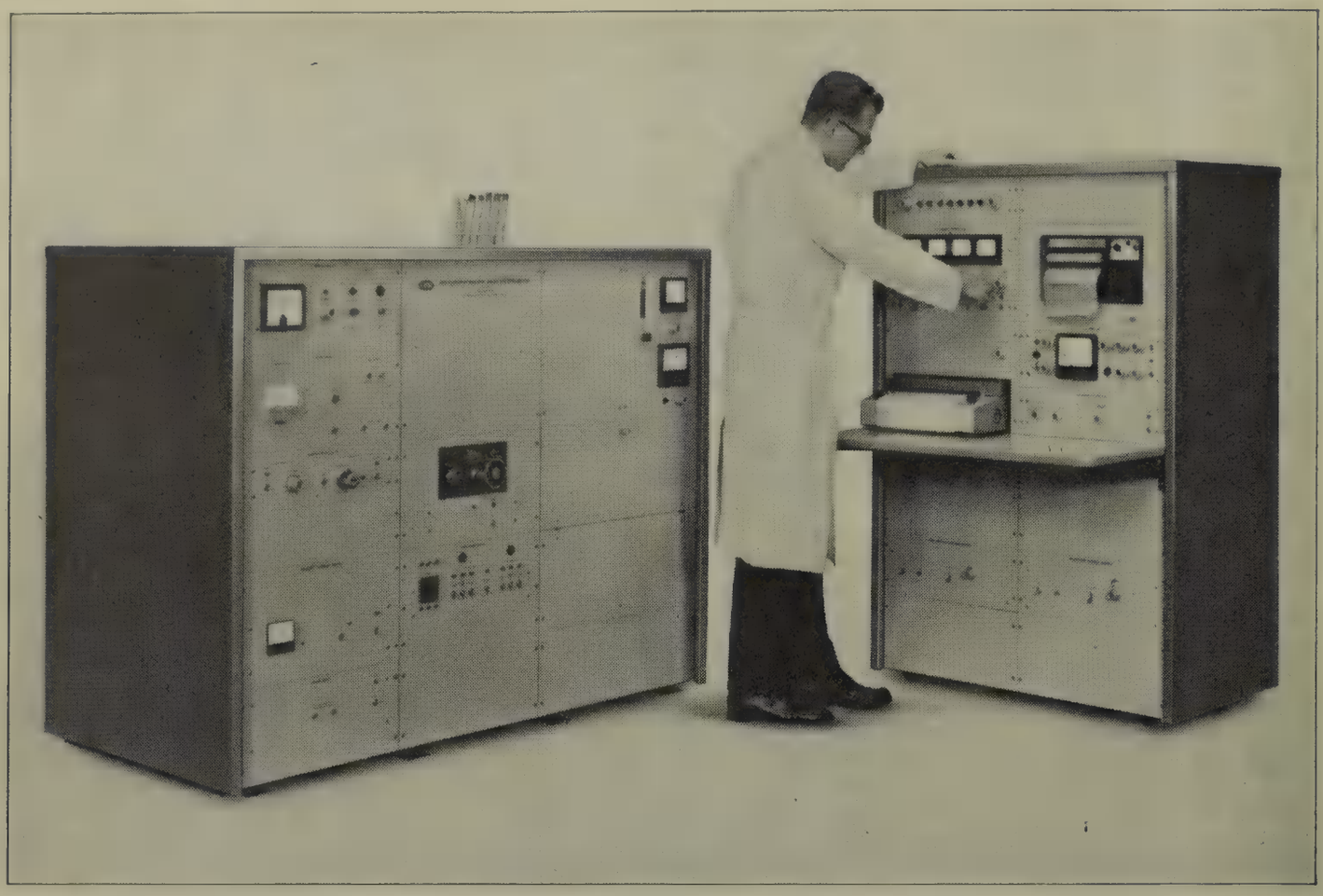

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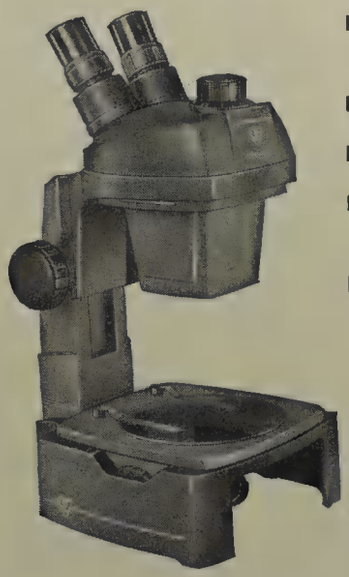

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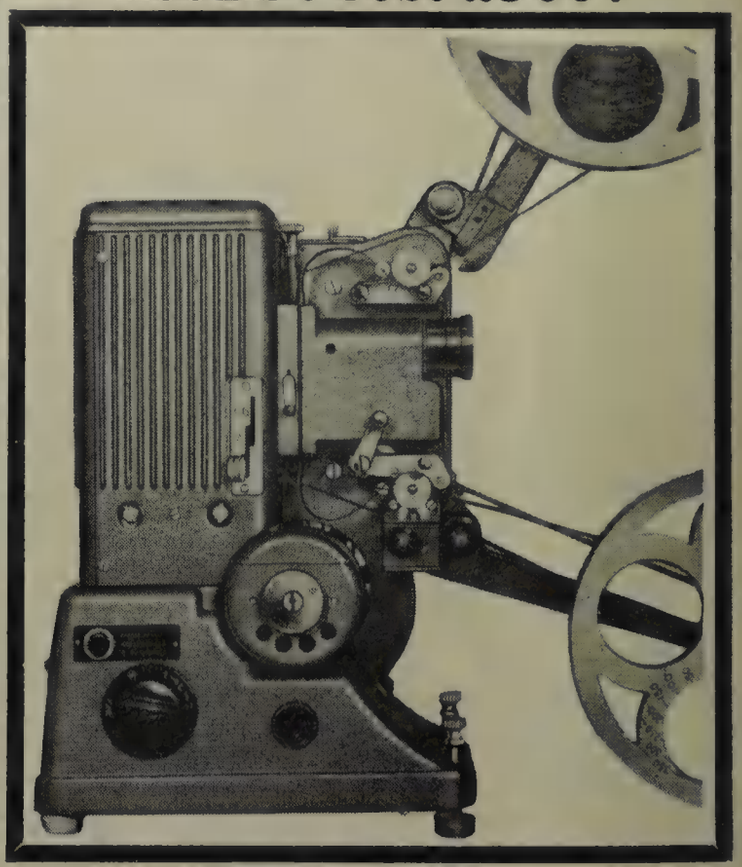

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